The Reproductive Ecology of Marine Turtles, *Chelonia mydas* and *Caretta caretta*, Nesting at Alagadi, Northern Cyprus, Eastern Mediterranean.

By

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Abstract

This thesis examines a wide range of subjects concerning the nesting and hatching of marine turtles, *Chelonia mydas* and *Caretta caretta* at Alagadi Beach on the north coast of the island of Cyprus (1993-1995). This is the most comprehensive study of its type to have been conducted in the Mediterranean, providing a unique opportunity to compare the two species nesting side by side. To date, 60 *C.mydas* and 99 *C.caretta* females have been tagged (1992-1996), allowing information to be gathered as to favoured remigration and inter-nesting intervals. Whilst *C.mydas* lay on average 2.4 clutches in any one season, *C.caretta* females lay, only 1.6. The latter require a greater period between laying subsequent clutches (13.5 days) compared to *C.mydas* (13 days). However, whilst some *C.caretta* females are able to lay in consecutive seasons and others favour an interval of 2 or 3 years, *C.mydas* females have only been recorded remigrating after 2 or 3 years. Females nesting at this site may require a greater remigration interval than the period of this study to prepare themselves for the reproductive process.

The inter-species variations in nesting frequency recorded in this study may be a consequence of the larger size of *C.mydas* (curved carapace length range; 78-106 cm) in comparison to the smaller *C.caretta* (curved carapace length range; 65-87 cm) and the strong relationship recorded between female size and mean clutch size. *C.caretta* nesting in Greece and Turkey have been shown to be significantly smaller than those nesting outwith the Mediterranean. This was also found to be the case for this species in Cyprus, and additionally *C.caretta* was found to lay significantly smaller clutches in comparison to those recorded in Greece. Again this may be a reflection of their smaller size, which may possibly indicate earlier maturation in this population or a shortage of nutrients in their feeding areas, leading to slower growth.

Although, in both species, larger females laid larger clutches, they did not lay significantly more clutches in one season than smaller females. However females, of both species, nesting earlier in a season were recorded to lay a greater number of clutches, but whilst larger *C.caretta* females were amongst the first nesters in a season, this was not the case for *C.mydas*. In the latter species, females showing a shorter inter-nesting interval between laying ultimately produced a greater number of clutches in a season. *C.mydas* females also oviposited into deeper nests and produced larger hatchlings than *C.caretta,* and both of these variables increased with the size of the female. No such relationships were recorded in *C.caretta*, however, nests of larger *C.caretta* females were found to have shorter incubation periods than smaller conspecifics. In addition, *C.caretta* nests which produced larger

hatchlings had a greater hatching and hatchling emergence success. Likewise in deeper nests a higher level of success was recorded.

The mean incubation period of *C.caretta* nests was shorter (47 days) than that of *C.mydas* (51 days) and those recorded elsewhere for these species in the Mediterranean. This is likely to be a result of the warmer temperatures prevailing in Cyprus. In some years, linear relationships were recorded as the incubation periods of nests decreased toward the end of the season, whilst in other years quadratic relationships were recorded, with nests laid at either end of the season requiring a longer incubation. Temperatures recorded by data-loggers ranged between 28.5-33°C in incubating *C.mydas* nests and 28.1-33°C in those of *C.caretta*. In nests of both species the majority of the incubation period was spent at temperatures above 29°C, which has been shown to be the pivotal temperature above which the sex ratio becomes increasingly skewed toward a greater proportion of females. If the temperatures in this small sample are typical and this is the pivotal temperature for these species in Cyprus, a greater number of hatchlings produced here will be female. However, seasonal and diurnal variations in the temperatures recorded in these nests may result in a different sex ratio than those achieved through artificial incubation at these temperatures. In light of the issue of global warming, interesting questions arise as to whether these species will be able to adapt to such climatic changes. Findings in this study, however, indicate that the timing of the onset of nesting is variable and may be governed by temperature and thus, in the event of global warming, these populations may shift their season so that it coincides with the correct temperatures for successful incubation.

The nesting behaviour of the two species is compared and the tagging procedure is critically examined as to its possible effects on subsequent behaviour and hatching success. *C.mydas* females spend on average twice as long in completing the nesting process than *C.caretta*. This is mainly a result of the longer time spent covering the nest. In *C.mydas,* a negative relationship was recorded between the time spent digging the egg chamber and the hatching and hatchling emergence success of the nest, possibly a result of females attempting to dig their egg chamber in an unsuitable substrate. Thus, not only is it more difficult to dig, but it is suboptimal for embryonic development, resulting in a lower level of success. This relationship was not recorded for *C.caretta,* however, individuals of this species laying larger clutches took a longer time to do so, a relationship that might be expected although was not recorded

for *C.mydas*. No significant effect of tagging females of either species was recorded in their resultant behaviour or in the success of the nest.

Inter-seasonal variations were recorded in the success of nests of both species. In 1993, a greater number (55%) of *C.caretta* nests hatched compared to *C.mydas* (48%), although in the two subsequent years of this study 77% and 91% of *C.mydas* nests hatched in comparison to 67% and 62% of *C.caretta* nests. Much of the inter-species variation was due to the higher level of predation of *C.caretta* nests by foxes and dogs, likely to be a result of their shallower nest and smaller nest-covering effort. This was a particular problem in 1995, which was reduced, in part, by placing protective screens above nests. In the same year, unhatched nests were investigated, and it was discovered that 20% of *C.caretta* nests had been flooded as a result of high waters.

In all years, nests of *C.mydas* hatched with a higher success than those of *C.caretta*. The main reason for this was that a larger number of unhatched *C.caretta* eggs contained dead embryos, indicating that this was not a problem of infertility but of unsuccessful incubation. Sand quality analysis showed that this lower success was not due to either the particle size or porosity of the substrate. However, positive relationships were recorded between the moisture content of the surrounding sand and the hatching success of *C.mydas* nests and between the moisture content of the sand and the incubation period of *C.caretta* nests. Insect infestation of hatched nests was also examined and found to be higher in *C.caretta* nests (23%) than those of *C.mydas* (9%).

Eggshell quality was examined in relation to its organic and inorganic components . Whilst the structure of eggshells of both species were in accordance with previous studies, toward the end of the season, shells were observed to contain a higher number of defects which may impede embryonic development. Similarly, levels of amino acids present in these shells varied within the season and between the two species, possibly a result of the depletion of resources and the varying diets of the two species.

Whilst this study has encompassed many variables associated with the reproductive biology of marine turtles nesting at Alagadi, it is the first of its type and has, as a result, has generated as many questions as it has answered. It is hoped that future studies at this site, and others in the Mediterranean, will provide answers to these questions, vital for these threatened species.

Acknowledgements

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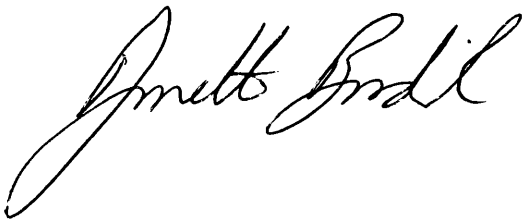
DECLARATION

I declare that the research described in this thesis has been carried out by myself unless otherwise acknowledged. All samples were brought into the U.K. under a CITES license.



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Chapter 1 - Introduction

1.1 Status and Distribution of Marine Turtles.

Marine turtles are found world-wide, nesting on beaches of tropical and sub-tropical seas. At least seven species of marine turtle exist world-wide today. These are *Chelonia mydas* (the green turtle), *Natator depressa* (the flatback turtle), *Caretta caretta* (the loggerhead turtle), *Dermochelys coriacea* (the leatherback turtle), *Eretmochelys imbricata* (the hawksbill turtle) *Lepidochelys kempi* (the Kemp's ridley turtle) and *Lepidochelys olivacea* (the olive ridley turtle). There is still some debate as to whether *Chelonia agassizii* (the black turtle or eastern Pacific green turtle), found nesting on the west coast of America, is a sub species of *C.mydas* or an eighth species of marine turtle. Bowen *et al.* (1992) found that mtDNA analyses did not support such a taxonomic distinction.

To be more specific, *C.mydas, C.caretta, D.coriacea* and *L.olivacea* nest extensively in tropical and subtropical areas (Groombridge 1982; Marquez 1990). *E.imbricata* are more restricted in their distribution, nesting mainly in the tropics, in areas associated with coral reefs (Marquez 1990). *N.depressa* are only found nesting in northern Australia (Groombridge 1982; Limpus 1995; Marquez 1990) and *L.kempi* are found nesting only in the Gulf of Mexico (Hildebrand 1982; Marquez 1990).

Both *L.olivacea* and *L.kempi* have been recorded as nesting in large masses or 'arribadas' although solitary nestings are also found (Hildebrand 1982; Marquez 1990). This behaviour is not seen in any of the other species and it has been suggested that it acts as a means to reduce predation levels of nests by satiating predators (Eckrich & Owens 1995).

Although nesting of these species is limited to the tropics and subtropics they may be found in more temperate areas in their efforts to feed. Indeed marine turtles have recently been added to the list of Scottish summer fauna due to their frequent occurrence in these waters (Gaywood 1996). Most of these sightings are of *D.coriacea* which, because of its large size can survive in cooler waters (Langton *et al.* 1996).

Trade in marine turtle meat, eggs, and in particular, 'tortoise-shell' (Groombridge 1982; Limpus 1995), has endangered many populations, and all marine turtle species are listed in Appendix 1 of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora).

The Mediterranean supports the nesting of two species of marine turtle; *C.mydas* and *C.caretta*. Studies of the populations of the latter have revealed them to be genetically distinct from the neighbouring Atlantic populations (Bowen *et al.* 1993; Laurent *et al.* 1994).

Other species, namely *D.coriacea*, *E. imbricata*, *L.kempi* and *L. olivacea* have been recorded in Mediterranean waters (Geldiay *et al.* 1982; Groombridge 1990; Sella 1982) although *C.caretta* may have been mistaken for the latter two. Most of these non nesting species are sighted in the western Mediterranean, and it has been suggested that they are likely to have been of Atlantic origin, coming into this area to feed. Both *C.caretta* and *C.mydas* are found nesting on many of the beaches of Cyprus (Broderick & Godley 1996; Demetropoulos & Hadjichristophorou 1995; Groombridge 1990; Groombridge & Whitmore 1989).

C.mydas is classified by the IUCN (International Union for the Conservation of Nature and Natural Resources) as 'endangered', and *C.caretta* as 'vulnerable' (Groombridge 1990). Sea turtles in the Mediterranean are protected by many international agreements, such as the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention), and the Convention on the Conservation of Migratory species of Wild Animals (Bonn Convention). However, the state of Northern Cyprus is recognised only by Turkey and so is not party to any of the above conventions.

1.2. Distribution of Mediterranean nesting populations.

Numbers of nesting females provide the only quantifiable estimate of the status of marine turtle populations. As neither males nor juveniles venture onto land, the sex ratio and absolute abundance of marine turtle populations can not be reliably measured. Previous estimates (Groombridge 1990) of the size of female nesting populations of marine turtles in the Mediterranean were calculated from beach surveys of nesting sites and assumed that for both species, each female laid approximately three clutches in a season.

Of the two species of marine turtle known to nest in the Mediterranean, *C.caretta* is present in larger numbers, with an estimated 2000 females nesting annually (Groombridge 1990). Most of this nesting occurs on the coast of Greece, Turkey, Cyprus and Libya, with some nesting in Tunisia, Egypt and the occasional clutch recorded in Israel and Italy

(Demetropoulos & Hadjichristophorou 1989; Groombridge 1990; Margaritoulis 1989; Schleich 1987; Venizelos 1993; Venizelos 1996).

The largest single nesting population of *C.caretta* is found in Greece, on the island of Zakynthos where some 300-700 females nest per season (Groombridge 1990). In addition the Peloponessus, Crete and Cephalonia, hold significant numbers of nesting females. The total annual population of *C.caretta* nesting in Greece is estimated at 1000 (Groombridge 1990).

In Turkey, most *C.caretta* nests are localised west of the city of Mersin. The total annual nesting population of *C.caretta* in Turkey is estimated at between 550 and 1000 females (Groombridge 1990). The population estimate in Southern Cyprus is 75 females nesting annually (Groombridge 1990), whereas in Northern Cyprus a larger population of between 150-200 females may nest annually (Broderick & Godley 1996). A survey of the eastern Libyan coast in 1995 has revealed a significant, but at present unquantified, nesting population (Venizelos 1996).

C.mydas nests are deeper than those of *C.caretta* and this is one of the reasons that the former is thought to prefer the slightly warmer temperatures of the eastern Mediterranean. It is now only found nesting in south east Turkey, Cyprus and occasionally in Israel (Baran & Kasparek 1989a; Broderick & Godley 1996; Demetropoulos & Hadjichristophorou 1995; Groombridge 1990).

The main nesting sites for *C.mydas* in Turkey are at Kazanli, Akyatan and Samandagi all lying south east of Mersin (Baran & Kasparek 1989b). In addition, a small number (less than 10) of *C.mydas* females have been recorded nesting at the Göksu Delta, situated just west of Mersin (Glen *et al.* 1997; Peters & Verhoeven 1992; van Piggelen 1993). At Kazanli an estimated 50 females nest each year (Carswell 1993), while at Akyatan an estimated 160 females were recorded in 1994 (Gerosa *et al.* 1995). Although Samandagi remains quoted in the literature (Groombridge 1990) as the third most important nesting beach for *C.mydas* in Turkey, no relevant nesting numbers have been published.

The island of Cyprus provides a number of suitable nesting beaches. In the south-west the Akamas Peninsula supports an estimated annual nesting population of 25-50 *C.mydas* (Groombridge 1988). The north of the island has scattered around its shores in excess of 30

C.mydas nesting beaches. Here, Godley and Broderick (1995a) calculated the annual female nesting population of *C.mydas* to be within the range of 100-150 turtles. The estimated total Mediterranean population of *C.mydas* is between 300-500 nesting females per annum (Groombridge 1990).

Hard evidence to validate assumptions of previous population levels does not exist. Studies of marine turtle populations in the Mediterranean are relatively new and most previous information is anecdotal. Thus, in the 1950's, there were reports of passengers on ferries crossing the Mediterranean witnessing what appeared to be 'shoals' of turtles in their hundreds (Groombridge 1990).

1.3. Life History

Marine turtles begin their lives on land where they emerge from their natal nest after 45-60 days incubation in the sand (Ehrhart 1982). On emergence they are highly active and head towards the sea, guided by both the slope of the beach and visual cues (Salmon & Wyneken 1990). On reaching the sea, they swim frantically to offshore waters in response to wave action (Salmon & Lohmann 1989). This swimming 'frenzy' continues for the next 3-4 days with all energy requirements being derived from the reabsorbed yolk (Lohmann 1992).

Once they reach the offshore waters, the hatchlings slow down and begin to feed on pelagic invertebrates and algae associated with drifting flotsam. It is thought that they remain in this environment for the next 3-5 years before moving inshore to feed in shallower waters (Lohmann 1992). It is estimated that only one hatchling in two thousand will survive to maturity (Lohmann 1992).

Hatchling marine turtles of all species are known to be omnivorous. Adult *C.mydas* are predominantly herbivores feeding on angiosperms such as sea grass (*Posodonia*) whereas adult *C.caretta* turtles are carnivores, feeding on fish, molluscs and crustaceans (Mortimer 1982b).

Recent evidence has been reported of *C.caretta* adults 'mining' for food in Australia. Preen (1996) observed and photographed three loggerhead turtles digging body pits in offshore sediment with their fore flippers and feeding on invertebrate organisms which they had

exposed by this process. The turtles left the site to surface for air, thereafter returning to their foraging site.

After a long maturation period, estimated at between 25-30 years (Frazer *et al.* 1994; Lohmann 1992), adult males and females migrate to the vicinity of nesting beaches. Here they feed and mate. It has been suggested that females mate with more than one male at the start of the season and store sperm from these matings for use in later egg production (Galbraith 1993; Gist & Jones 1989). During a nesting season females have been recorded as laying between 1 and 10 clutches at intervals of 10-15 days (Carr 1982; Hughes 1974). They may nest every 1-3 years, although variation amongst species, individuals and populations exists.

Females have been documented as being nest site philopatric, both within and between nesting seasons (Mortimer & Porter 1989). However, no conclusive studies have been conducted to wholly support the theory that marine turtles exhibit natal philopatry. One study by Grassman (1993) showed that six month old green turtles responded to chemicals to which they were exposed as embryos. This would certainly support the theory of chemical imprinting but not necessarily the theory of homing.

Estimates of the maximum life span of marine turtles range from 60-120 years (Lohmann 1992; Lohmann & Lohmann 1996). Such a time scale however makes confirmation of data extremely difficult. Neither is it known at what stage individuals are no longer able to reproduce. Studies of carapace growth rings have proved inconclusive at ageing turtles, however, other studies such as those by Zug *et al.* (1986) have shown that growth lines in the humerus of *C.caretta* may be age related. Large amounts of data, however, are required to substantiate such estimates and must rely on material collected from stranded turtle carcasses or biopsies from captured turtles.

When choosing her nest site, many factors may be selected for by the female, such as slope of offshore approach (Mortimer 1982a), slope of the beach, sand type (Mortimer 1990), depth, moisture and temperature as well as levels of covering debris. A gradual slope is thought to be preferred for ease of nesting and to reduce possible wash-over from the surf, since inundation reduces hatching success. McGehee (1990) found that hatching success was highest in nests of moisture levels of 25% and lowest at 100%. Incubation periods also

lengthened with an increase in moisture. However total flooding of a nest can suffocate, or cause critical cooling of developing embryos (Kraemer & Bell 1980; McGehee 1990). Mortimer (1990) analysed sand particle size on *C.mydas* nesting beaches and showed that most of these beaches around the world had sand particle diameters of between 0.2-1.0 mm. The temperature of a nest is also critical for both development and sex determination. Harry and Limpus (1989) demonstrated that *C.caretta* eggs incubated at 26°C in Australia all developed as males, whilst those incubated at 32°C resulted in female hatchlings only. Normally, it is thought that most nests have a temperature intermediate to these extremes.

An additional factor which may affect hatching success is eggshell quality. Poor quality may impede the passage of gases and water across the eggshell. In addition, fungal or microbial invasion of the egg and shell can occur, blocking valuable diffusion space and competing with the developing hatchling for resources (Solomon & Tippett 1987).

The nesting activities of the two species are markedly different. *Chelonia mydas* digs her body pit with all four flippers. Upon reaching a depth of up to 50 cm below the level of the surface of the sand, she then digs her egg chamber, using only her hind flippers (extending a further 30 cm in depth). At this stage she may abort her activities, due to unsatisfactory conditions, and either go on to attempt to nest elsewhere on the beach or return to the water. If she builds the egg chamber successfully she will lay approximately 50-150 eggs per clutch. She then covers the egg chamber, packing sand down with her hind flippers, and covering the body pit with sand, using all four flippers as she moves forward, often a whole body length or more. This act disguises her actual nesting position. *C.caretta* females dig a much shallower body pit and hence the egg chamber also tends to be shallower. *C.caretta* expends less effort on covering the nest and little attempt is made to disguise its presence.

It has been suggested that both adult and hatchling turtles use both current patterns and magnetic cues to orientate and migrate with respect to their nesting and feeding grounds (Lohmann 1991; Lohmann 1992). Recent research on the oceanic movements of adult *D.coriacea* has shown that they follow distinct migration corridors (Morreale *et al.* 1996). They suggest that whilst most conservation efforts so far have focused on nesting and foraging areas, attention should now be given to these migration corridors. Limited research has, so far, been carried out on the movements of marine turtles within the Mediterranean although it is thought that they do not leave this area.

1.4. Threats to marine turtles

1.4.1. Impact of tourism

Over the last decade, tourism in countries with warm climates has escalated. With this comes increased beach development and use. Many factors associated with tourism are detrimental to nesting marine turtle populations (Shabica 1982). Beach parties, noise, and excessive physical presence may disturb adults and lead to the abandoning of nesting attempts (Arianoutsou 1988). Tourists may also directly disturb turtles by coming at night to observe their nesting activities.

Human activity on nesting beaches brings with it a number of problems. Traffic on the beach causes compaction of the sand, making emergence for hatchlings difficult or actually crushing them as they near the surface. Ruts left from tyre tracks act as trenches which hatchlings have problems negotiating and in which they may overturn and desiccate or be trapped, increasing their chances of predation (Witham 1982).

The use of beach umbrellas can cause piercing of eggs. Both umbrellas and sun beds may also cause critical cooling of nests through shading, altering the development and sex ratio of the nest, or even arresting development completely. If left on beaches overnight, these objects may also act as obstructions to nesting adults (Warren & Antonopoulou 1990).

With the increased usage of a beach, comes an increase in litter. As well as being hazardous to the turtles, organic debris may attract scavengers to the beach and hence increase predation levels of eggs and hatchlings. In addition, an increase in sewage output, often inadequately treated, is associated with beach development and this may affect the benthic flora and fauna, crucial for the turtles diet.

The use of powered speed boats has in recent years caused the deaths of many turtles (Arianoutsou 1988). The Greek island of Zakynthos is the most important loggerhead nesting site in the Mediterranean and at least nine turtles were recorded as having been killed by speed boats during the 1993 nesting season (Venizelos 1993).

Hatchling turtles are believed to be attracted towards light and move faster under brighter conditions. This aids them in reaching the relatively bright surf since they are repelled by a broken horizon, such as vegetation or rocks at the back of the beach. Lights from beach

developments may disorientate both adult and hatchling turtles, especially on cloudy nights (Salmon & Witherington 1995; Witherington 1992). In Florida, an example of this was recorded by McFarlane (1963) where in one incident 90 out of 115 hatchlings were run over by vehicles as a result of being attracted to lights at the back of the beach. Witherington (1992) showed that wavelength of light is also important. Lighting beaches with low pressure sodium vapour lamps (LPS) with longer wavelength emissions had little effect on nesting turtles whereas mercury vapour lamps, with a higher proportion of short wavelength significantly reduced the number of green and loggerhead turtles emerging and nesting within the lighted study areas. Similarly hatchling turtles were attracted less to LPS luminaries (Witherington & Bjorndal 1991).

1.4.2 Sand Extraction

The removal of sand from a nesting beach can be detrimental to the dune and beach structure and ecology and thus to both turtle and tourist activity. As sand is taken away from the dune, beach sand blows away from the shore, having no barrier to prevent its displacement by onshore winds. This results in an overall loss of sand from the beach, leaving it stony and progressively more shallow for turtles to nest. Once this situation has occurred it is very hard to reverse the effects. Coastlines themselves can be altered as a result of such activities (Shabica 1982).

1.4.3 Mortality from fisheries

Incidental catch as a result of fishing activities is the cause of many marine turtle fatalities around the world. In many cases live turtles are released after capture, however if long-lines have been used, hooks may be embedded in the turtles mouths and survival may be low. In the U.S.A., Turtle Excluder Devices (TEDs) are a legal requirement on shrimp trawlers resulting in the reduction of incidental catches (Henwood & Stuntz 1987). Such equipment and control would be difficult in the Mediterranean where so many countries are involved, many of which are relatively underdeveloped and lacking in fisheries/coastguard patrol vessels and enforcement.

In the Mediterranean, an estimated 50,000-100,000 mature and young turtles are caught each year on longline hooks and in nets set for fish (Groombridge 1990; Venizelos 1991a). This is not always fatal, some may be released alive. There is little trade in marine turtle products in this region, however, carapaces are sometimes sold complete, or in part, as tourist souvenirs. In some parts of the Mediterranean, such as Tunisia, turtle meat is used for

domestic consumption and thus fisheries for turtles may still exist (Groombridge 1990). It is feasible however that many of these turtles are not of Mediterranean origin and may include migrant feeders from the western Atlantic populations.

1.4.4. Disease

Among the new threats to turtles, is the increasing incidence of fibropapilloma disease (Hutchinson & Simmonds 1992). Affected turtles exhibit large external tumours which may impair movement or grow across the eyes or mouth inhibiting feeding, breathing and vision. This disease is documented mainly for *C.mydas,* in which it is most common, and is thus commonly known as 'green turtle fibropapilloma disease' (GTFP). Having originally been documented in 1938 as a very rare condition, GTFP has now reached epidemic proportions in Florida. In the Mediterranean, the occurrence of GTFP has not as yet been recorded. Suggestions as to its aetiology range from viruses, parasites and algae to pollution, either directly or by causing stresses and so weakening the immune system (Balazs & Pooley 1991). Recent evidence suggests that the cause of this disease is indeed a virus (Herbst *et al.* 1996). Koga and Balazs (1996) reported that in stranded *C.mydas* turtles in Hawaii the sex ratio was 1:1 although the sex ratio of stranded turtles with tumours was biased towards females, suggesting that they may be more susceptible to this disease.

1.4.5. Pollution

Marine predators ultimately accumulate much of the persistent pollution such as PCB's in the marine environment through the food chain (Conner & O'Dell 1990). However, few investigations have been made into the concentrations of such compounds in marine turtles. Davenport *et al* (1990) found that tissue taken from a male leatherback stranded in Wales had fairly high concentrations of PCB's in the fat when compared to fish, although not as high as concentrations found in marine mammals and birds. Nevertheless, the accumulation of pollutants may be detrimental regardless of their concentrations.

Some of the PCB's in marine ecosystems may be derived from the breakdown of ever increasing amounts of plastic debris found in the world's oceans and seas. Litter or marine debris is not only aesthetically detrimental to the marine environment but also ecologically damaging. Synthetic fishing nets discarded by fishermen, plastic bottles, bags and packing bands can entangle or be ingested by many marine animals such as seabirds, mammals, fish and turtles (Dixon & Dixon 1981). It has been suggested that turtles may mistake flotsam for edible items, thus plastic bags with their jellyfish like appearance are frequently ingested

(Gramentz 1988). Balazs (1985) reported 79 cases in which turtles had ingested materials such as plastic, metal and tar balls and 60 instances of entanglement. Of these 95% had occurred since 1970. Allen (1992) similarly reported a loggerhead sea turtle washed ashore in California. Death was attributed to the ingestion of several plastic and wood pieces, possibly causing faecal impaction. Turtle hatchlings spend 3-5 years during the juvenile pelagic stage drifting on flotsam. It is here that they will come into contact with buoyant debris. Hatchlings have been documented washed ashore containing tar pellets and plastic beads similar in size and shape to the floating vesicles that fragment from *Sargassum* plants, which are also thought to be part of the diet at this life stage (Carl 1987).

The Mediterranean is surrounded by industrialised countries and has very busy shipping lanes as well as having a water circulation pattern which circulates and retains floating material (McCoy 1988; Morris 1980). Water exchange between the almost land locked sea and Atlantic is slow and limited. Stratification concentrates pollutants in particular areas as currents mass litter in particular regions. Thus, floating litter accumulates in these areas and storms at sea break up the litter masses and deposit them on coastlines (Golik & Gertner 1992; Golik *et al.* 1988; Stansell 1990).

The increase in pollutants also results from the increase in tourism, bringing with it a heavier burden of waste. Because the Mediterranean is an essentially enclosed sea, it is rapidly affected by toxic effluents and dumped waste (Gabrielides *et al.* 1991). It is generally agreed that it is the most polluted sea in the world. The extent to which debris on the beach affects nesting and hatching turtles is unknown. That it does have some effect on both adults and hatchling is however certain (Venizelos 1991b).

There are many conventions for the control of marine pollution in the Mediterranean sea. However, as not all Mediterranean coastal countries are members of these treaties it is difficult to control such marine problems.

1.4.6. Predation

Adult turtles were thought to face little threat from predation although recent evidence suggests that the endangered monk seal (*Monachus monachus*) may be preying upon adult loggerhead turtles in the Mediterranean sea (Margaritoulis *et al.* 1996b). In Turkey the golden jackal (*Canis aureus*) has been reported attacking and killing adult loggerhead turtles (Peters & Verhoeven 1992; Peters *et al.* 1994b). On the other hand, turtle hatchlings face

many threats whilst in the nest, on the way to the sea, from foxes, feral dogs, ghost crabs (*Ocypode cursor*), and in the water, from birds and fish respectively (Gyuris 1994; Stancyk 1982; Witherington & Salmon 1992). In Australia Gyuris (1994) reported that 31% of hatchlings were predated by fish whilst dispersing from the beach across a reef.

1.5. Previous surveys in Northern Cyprus.

In 1988, Whitmore and Groombridge conducted the first scientific survey of the nesting beaches in Northern Cyprus (Groombridge 1988; Groombridge & Whitmore 1989) and estimated there to have been 96 *C.mydas* and 122 *C.caretta* nests during the 30 day period of study. They highlighted two stretches of beaches of significant importance to nesting turtles and recommended their protection by local authorities. These were the Chelones beaches, situated on the north coast of the Karpaz Peninsula, and Alagadi Beach, the site of this study.

In 1992 the Society for the Protection Of Turtles in Northern Cyprus invited the University of Glasgow to conduct a survey of the nesting beaches in Northern Cyprus. In that study 84 beaches were surveyed with 65 recorded as having turtle activity and a minimum of 219 nests (Godley & Broderick 1992). Each year thereafter, a group from Glasgow has returned to Northern Cyprus and has recorded 571, 980 and 876 nests in 1993, 1994 and 1995 respectively (Broderick & Godley 1993; Broderick & Godley 1995; Broderick *et al.* 1996; Godley & Broderick 1994; Godley & Broderick 1995b).

The fieldwork for the investigations in this thesis was carried out in conjunction and with field assistance from Glasgow University Turtle Conservation Expedition (GUTCE).

1.6. Aims of this study

Few detailed studies have been conducted on Mediterranean marine turtles, in particular *C.mydas,* due to its limited distribution. In addition few beaches in the Mediterranean have both *C.mydas* and *C.caretta* nesting on them. Northern Cyprus is a particularly suitable site for the study of marine turtles, since it is the location of the majority of beaches used by both species, enabling a comparative study to be undertaken. Alagadi beach, the site of this study, is the main nesting site for marine turtles in Northern Cyprus.

In any study of marine turtle nesting populations, it is first necessary to describe the general nesting ecology. Following this the various factors influencing their behaviour and the resultant success of their nests must be examined. These include environmental conditions on the beach, incubation conditions within the nest, behaviour and activity of the female and condition and quality of the eggs. The aim of this thesis is to consider all these aspects. However with such a broad subject, not all areas will be examined or discussed to the same depth. In addition, due to the endangered status of these species some areas were not explored as the interference necessary was not deemed necessary.

At present, Northern Cyprus is one of the few remaining places in the Mediterranean that has nesting turtles and has not been developed for tourism. With the correct management before such development occurs, which is inevitable, these nesting populations of marine turtles can be protected.

Chapter 2 - General Methodology and Study Site

2.1. Study site

Cyprus is situated in the eastern Mediterranean (see Figure 2.1), close to the coast of Turkey, at 35°N, 33°E. Since 1974, it has been divided politically, with the Turkish Cypriot community in the north and the Greek Cypriot community in the south. The human population in the north of Cyprus presently stands at over 170,000 and is steadily increasing. Over the last five years the tourist industry has risen by more than 100%. Northern Cyprus now has over 100,000 visitors every year. Mean daily air temperatures during the summer months, of May to October range from 27 - 36°C, with a recorded 400 hours of sunshine per month.

Field work was conducted in Northern Cyprus during the nesting seasons (May - October) of 1993,1994 and 1995 on Alagadi beach. The latter area was identified as the major nesting site of marine turtles in Northern Cyprus during preliminary work in 1992. This beach is situated on the north coast of the island, approximately 16 km east of Girne (Kyrenia) (see figure 2.2.). It consists of two bays, of 1.4 km and 0.8 km respectively separated by a rocky headland. The beach has also been designated for public use and is heavily used during the summer months.

In 1994, the Department of Environmental Protection (DoE) in Northern Cyprus instituted a policy of closing Alagadi beach to the general public from May to October between the hours of 20:00 until 08:00. Although some people are still unaware of this fact and come onto the beach at night, disturbance to marine turtles at Alagadi beach has decreased dramatically. Also in 1994, a small beach bar was built at the back of Alagadi beach with toilet and shower facilities and this has lead to an increase in human pressure during the day. However, in 1995, the DoE placed signs at all entrances to the beach asking users to follow a code of conduct in order to protect incubating marine turtle nests. In addition, areas of the beach are now cordoned off so that sunbathers are prevented from sitting directly above nests.

The work involved nightly surveys during the nesting season and day time dawn surveys throughout the hatching season for three years from 1993-1995. Additional tagging data and overall nesting and hatching numbers are also included for 1992 and 1996. The latter data were collected by members of Glasgow University Turtle Conservation Project.

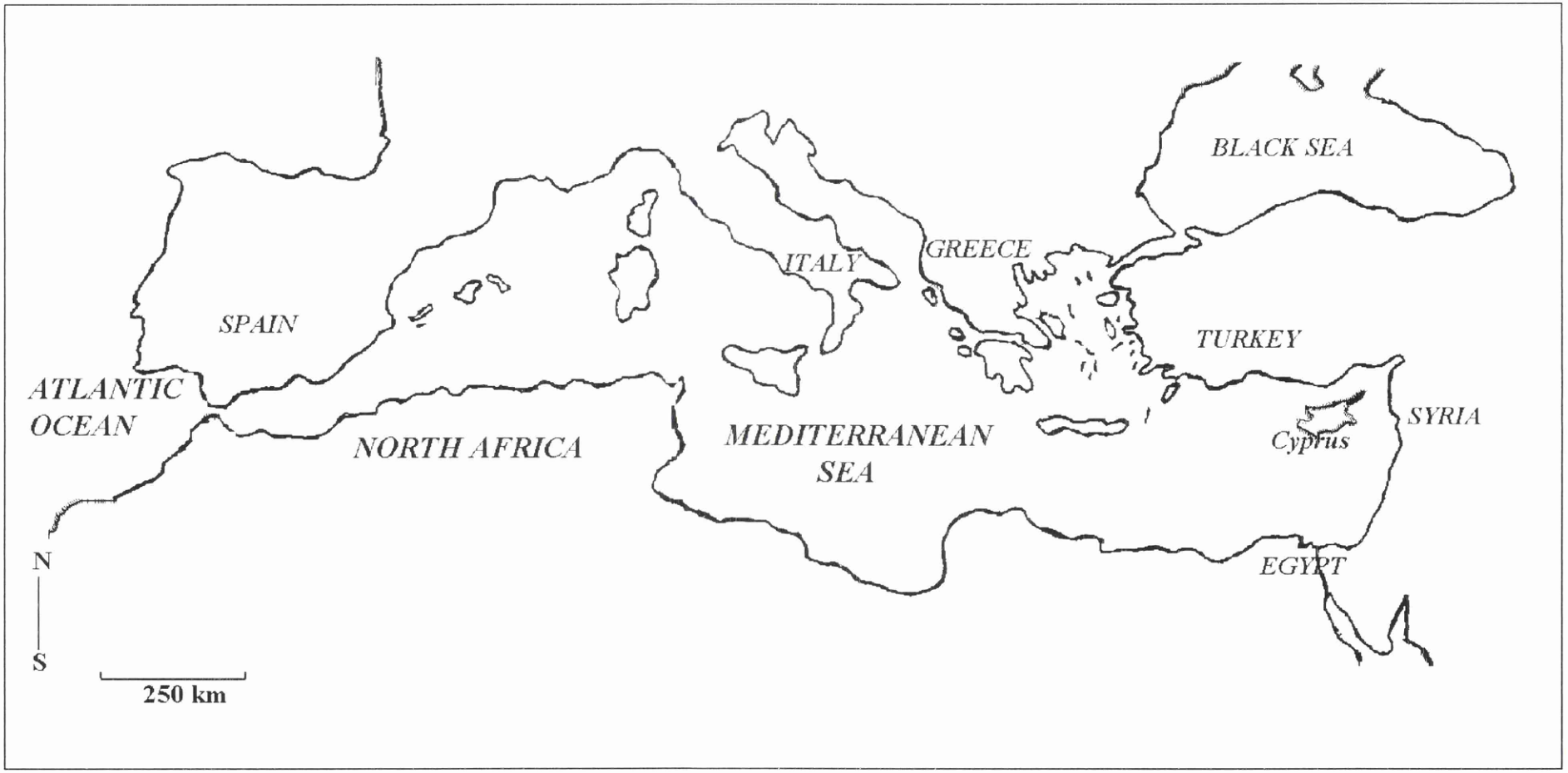


Figure 2.1. The Mediterranean, illustrating the proximity of Cyprus to the coast of Turkey.

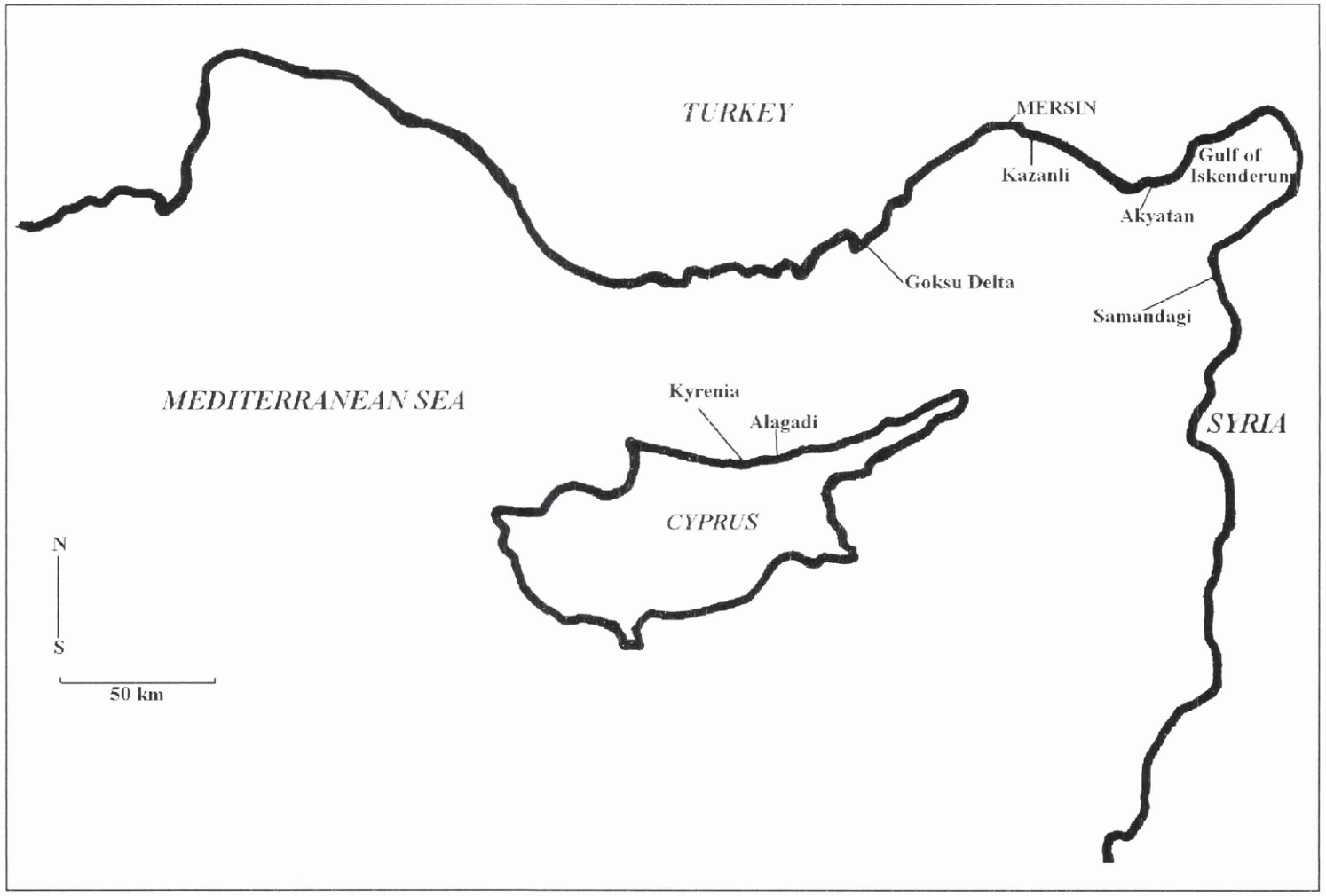


Figure 2.2. A closer view of Cyprus showing the site of this study, Alagadi, and other *C.mydas* nesting sites on the east coast of Turkey.

2.2. Nesting data

Alagadi beach was divided at 50m intervals by a line of posts placed at the back of the beach, to permit accurate positioning of turtle activities. Throughout the nesting season (late May/June- mid/late August) the beach was surveyed from dusk until dawn every night by 6-8 individuals in groups of two. As little artificial lighting as possible was used, until a fresh track or adult turtle was encountered. If the female was present, minimum torch light was used. The stage of activity was noted and the times of the onset of all subsequent activities recorded according to the following categories;

Ascent- The period of time from the female leaving the sea until she starts to dig.
Digging body pit - all four flippers are used to dig a large pit.
Digging egg chamber - a deeper tubular chamber is dug with the rear flippers only.
Laying - no flipper movement, usually the female lowers and raises her posterior end. On closer viewing eggs can be observed as they are being deposited.
Covering egg chamber - with rear flippers only, sand is packed on top of the eggs
Covering body pit - sand is thrown with all four flippers to cover the original body pit.
Descent - The female leaves the nest site and returns to the sea.

During the laying phase of the nesting process species identification was confirmed and measurements of curved carapace lengths and widths taken to the nearest centimetre. Turtles were examined for distinguishing features and existing tags.

If a female was not observed, but sand disturbance encountered, the species and activity were assessed using the criteria of track and nest pit morphology. *C.mydas* makes a symmetrical crawl track and nesting attempts are usually associated with a deep pit and a large amount of sand disturbance. When nesting has occurred, a great deal of covering up activity is evident. *C.caretta* makes a markedly asymmetrical crawl track and a shallow nest pit with only a small degree of associated disturbance.

Each activity was assessed and classified into the following behavioural categories:

a) 'successful nesting' (N) - recorded when a crawl track was visible leading to an area of disturbed sand where digging and covering had occurred.

b) 'false crawl attempted nesting' (FCA) - recorded when some disturbance, if only slight, occurred but no covering up was apparent.
c) 'false crawl U-turn' (FCU) - recorded when a turtle made no nesting or digging attempts, simply crawled on the beach and returned to the sea.

Following oviposition, a small numbered plastic tag was placed in the nest above the egg chamber. If the adult had not been previously tagged, plastic tags (Jumbo tags / Supertags, Dalton Supplies Ltd.) were placed through the trailing edge of both fore flippers, as far from the distal edge of the flipper as possible (position recommended by Limpus 1992). In 1992 and 1993 green coloured flipper tags were used (Jumbo tags), in 1994 yellow tags (Supertags) and in 1995 blue tags (Supertags).

The reasons for changing from Jumbotags to Supertags in 1994 was that the Supertag displayed a greater strength in the pin, making it less likely to bend upon application. The non pin part of the Supertag was also of a more flexible type of plastic, and it was thought that this may offer less resistance and thus less chance of tag loss. Despite these precautions, in 1996, females that had been tagged in 1994 with these new Supertags, returned to nest and in some cases the numbers on the tags had almost completely worn away, making them unreadable. New tags are being discussed for use in 1997.

Once the turtle had returned to the sea, the position of the activity was recorded by measuring the distance from the nest to the two nearest marker posts using 50m tape measures. The date of activities was recorded as the date prior to midnight on the night of the patrol. Inter-nesting intervals were recorded between subsequent clutches of multiple nesting females.

2.3. Relocated nests.

In some instances, nests are laid too close to the waters edge, and it is considered necessary to move them to a safer position on the beach to prevent inundation. In these cases, if the nesting was witnessed, the female was allowed to cover over her nest and return to the sea, after which the nest was excavated and the eggs removed. A new, artificial nest was dug higher up the beach to set specifications of mean nest depths, and eggs placed into the new nest in their original order. The positions of the new and old nest sites were recorded.

Occasionally, nests are exposed by high water and if salvageable they were relocated higher up the beach. Relocation was only considered as a last resort where a nest was otherwise considered 'doomed', as hatching success has been shown to be reduced by this process (Whitmore & Dutton 1985).

2.4. Predated nests.

During nesting and hatching surveys any evidence of predation was recorded. This is usually apparent because of the presence of eggshells scattered over the surrounding area. Predator signs such as fox and/or dog tracks were also recorded. Attempts were made to match the nest to an already recorded one. This was done by either; calculating the stage of embryo development and hence the age of the nest from remains; measuring the nest to the markers on the beach; or finding the nest tag. Where possible any potentially viable eggs were salvaged and reburied.

When a nest had either been transplanted or partially predated, a screen was put into place in an attempt to protect it from further predation. This took the form of a wire grid mesh (10 cm) which was placed beneath the sand at a depth of 15-30 cm and staked out above the clutch. Holes in the screen were large enough for hatchlings to pass through but small enough to retain strength and prevent predators from digging through, thus protecting the clutch.

2.5. Hatching data

From mid July until mid October the beach was also surveyed at dawn for signs of hatching. This phenomenon is easily detected from the numerous small turtle tracks which create a mottled effect over the dry sand. Hatchling tracks were traced back to an epicentre, a slight depression in the sand which occurs as the sand percolates down the nest column to fill the space previously taken up by the eggs/hatchlings.

The activity was recorded under the following headings:
a) 'hatched nest' (H) - described as above with no apparent disturbance.
b) 'hatched and predated' (HP) - as with a predated nest although hatchling tracks were apparent.

If a nest was recorded as hatched, but with less than 20 hatchling tracks apparent, the nest was left, caged for protection and revisited the following morning. If complete hatching was thought to have occurred, the position of the activity on the beach was noted and the nest was then excavated by hand. Care was needed at this point, as live hatchlings were frequently found in the nest column. The depth from the surface of the sand to the top of the egg chamber was recorded, and the shell debris and unhatched eggs removed. The depth to the bottom of the egg chamber was then recorded. Live hatchlings were counted and straight carapace length and width measured, to the nearest 0.1 mm, using callipers. The hatchlings were then released and allowed to crawl from the nest site to the sea unaided, whilst being carefully monitored to avoid the risk of predation by ghost crabs or overturning because of debris or ruts on the beach.

A count of the eggs removed from the nest was made. It is possible from the shape and colouration of the shell to assess if there is a live embryo in the shell. If this was the case the eggs were reburied or artificially incubated in buckets. Unhatched eggs, clearly not containing a live embryo were incised and categorised into the following:

a)'undeveloped' - unhatched eggs with no sign of gross development.
b)'non-viable' - these were non-yolked and often much smaller. They are thought to occur at the start or end of the lay, possibly due to developmental abnormalities or to act as packaging and so may protect the viable eggs of the clutch.
c)'dead-in-shell' - eggs containing a dead embryo which were staged according to Miller (1985).

From hatchlings or 'dead-in-shells' the species of the nest could be confirmed. All shell debris was removed from the beach to avoid confusion and discourage predators. Hatchling tracks were raked over and the position of the activity was recorded.

By knowing the position of the nest and/or the nest tag number the date on which the nest was laid could be evaluated. The incubation period could then be calculated as the number of complete nights from the laying of the clutch to the emergence of the first hatchlings. As with nesting data, the date was taken as that before midnight for the nights activity.

Clutch size was calculated from a count of unhatched eggs and hatched egg fragments. The hatching success of an individual nest was calculated as the percentage of eggs of the clutch which successfully hatched. The emergence success of a nest however takes into account the number of hatchlings which failed to emerge from the sand alive. Thus, this is a measure of the percentage of hatchlings which successfully emerged from the nest rather than from the egg.

2.6. Sample collection

Egg shells from all the above categories were collected for egg quality analysis, together with sand samples from the egg chamber of each nest. Sand samples were analysed at the study site for moisture and porosity characteristics and returned to the laboratory for particle analyses. In addition, any insect larvae found infesting nests were collected and reared for identification.

Chapter 3 - Nesting Periodicity and Site Fidelity of *Chelonia mydas* and *Caretta caretta* at Alagadi.

3.1. Introduction

The size of a population, at any one time, is a reflection of births versus deaths and is relatively easy to calculate when the species under consideration is retained in its habitat. Marine turtles do not fall into this category and so the population status has to be estimated using a variety of direct and indirect measurements (Crouse & Frazer 1995). Accumulating this information is a long term commitment. Yearly fluctuations in nesting numbers are common in marine turtles, particularly *C.mydas* (Bagley *et al.* 1996; Balazs 1996; Ehrhart *et al.* 1996; Limpus 1996b). In contrast, *C.caretta* displays a certain stability in its annual activities (Ehrhart *et al.* 1996).

Marine turtles have been acknowledged, by many scientists, to return to nest on the same beaches year after year (Carr 1975; Mortimer & Porter 1989; Papi & Luschi 1996). However, the absolute level of such fidelity has rarely been documented; thus Johnson and Ehrhart (1984) suggest that the phenomenon relates to a stretch of coastline and if only one beach is present in that area then selection for the latter will be high. Environmental influences will undoubtedly cause a change in the topography of nesting beaches and hence alter the emergence pattern. In South Carolina, Tarlbert *et al.*(1980) calculated the average distance between subsequent nesting emergences as 3.2 km. In Florida, this value was given as 1.9 km for *C.mydas* (Johnson & Ehrhart 1994). Recent technological advances support the theory of site fidelity. Schroth *et al.* (1996) provide evidence of genetic separation of *C.caretta* populations with respect to different coastal areas of Turkey.

The remigration intervals for *C.mydas* and *C.caretta* have been shown to vary both within and between populations with some showing cyclical patterns and others not. The most common remigration interval recorded for *C.mydas* is 3 years (Hughes 1982) with intervals of between 2 and 11 years also on record (Limpus *et al.* 1994). At the Great Barrier Reef, Limpus *et al.* (1994) found that the mean interval between *C.mydas* females nesting was 5 years, with 15% of females changing rookeries between seasons and nesting within a 60 km radius of their original rookery. *C.caretta* females have been recorded remigrating after intervals of between 1 and 9 years although most studies suggest that a 2 year interval is favoured (Davis & Whiting 1977; Dodd 1988; Hughes 1982; Richardson & Richardson 1982).

In a study of *C.caretta* nesting on the Greek island of Cephalonia, Hays and Sutherland (1991) reported that females re-migrated to nesting beaches after a mean period of 2.56 years. After 4 years, only 43.5% of the females tagged in the initial year had returned to nest. However, 80% of females laying more than one clutch in the initial year returned within the four year period. They suggested that these females showed a greater degree of site fidelity than other females who may also have been laying at other nesting sites. In a study based on one loggerhead turtle, Hays *et al.* (1991) reported the female in question nesting four times in the nesting season at different sites on the island of Cephalonia. Similarly, Margaritoulis (1997) reported a loggerhead turtle nesting at two sites in Greece during one nesting season. These two sites were more than 500 km apart.

A large variation has been reported with regard to the mean number of clutches any female will lay in a given season, both temporally and spatially. The most common estimate for *C.caretta* is 1-3 nests (Dodd 1988; Richardson & Richardson 1982) however in some areas such as Tongaland this figure is higher at 4-5 clutches per female (Hughes 1982). *C.mydas* may lay between 1-10 clutches in one season, laying on average 2-5 clutches per annum (Groombridge 1982; Marquez 1990).

Addison (1996) plotted the frequency of inter-nesting intervals of *C.caretta* females nesting in Florida between 1985-1994 and recorded intervals ranging from 9-70 days with peaks observed between 9-17 days and 20-27 days. He suggested that nestings had been missed accounting for this bi-modal distribution and adjusted the figures according to this information. It was previously thought that females nesting in this area laid an average of 3 nests per season, whereas the research by Addison (1996) indicates that females laid, on average, 3.9 clutches, hence there may be 25% fewer nesting females in the population that was thought.

Unless some form of remote telemetry is used or every nesting attempt on all beaches witnessed, it can never be known for certain whether females are nesting on other beaches during or between nesting seasons. Without detailed information on remigration intervals and the number of clutches laid in a season, it is very difficult to estimate the size of the breeding population and ignorance of the natural sex ratios and age structure prevents extrapolation to the population as a whole (Meylan 1982).

Although studies on marine turtle nesting populations in the Mediterranean have been carried out, few data have been published and the available data are particularly poor owing to the limited distribution and low numbers of *C.mydas*. The population estimates for other nesting grounds in the Mediterranean are given in chapter 1. In this chapter remigration and fidelity of nesting females is examined. In addition, a comparison of different methods of estimating populations is presented, using information on the number of females tagged, inter-nesting intervals and clutches laid.

Although the present study was conducted over the three years 1993-1995, data are also included for 1992 and 1996 so that the reader may gain a more comprehensive understanding of the populations nesting at Alagadi.

3.2. Methodology

Field methods used in the collection of these nesting data are described in chapter 2.

Data are presented on the mean number of clutches laid and the intervals between clutches. In addition, by plotting frequency histograms of inter-nesting intervals the durations recorded between subsequent nestings were examined. From this information it was apparent that some females were recorded as re-nesting after intervals corresponding to two or three inter-nesting periods. This suggested that they had laid clutch(es) during this interval which had not been observed. The observed number of clutches a female was recorded laying was therefore corrected to take these nests into account. This value is referred to as the 'estimated' value for the mean number of clutches laid per female and is used in calculating population estimate 3 below.

Repeatability analysis was used to examine how much variation in the inter-nesting intervals was a result of individual variation in females. For this, only complete records were used. This was done by treating individual females as different samples and comparing them using a one way ANOVA. If significant, a repeatability coefficient was calculated using the method described by Harper (1994).

Information is given on the proportion of nests that were observed and similarly the proportion that were attributed to known females. Using this information the mean number of clutches recorded per female was adjusted to take account of any unattributed clutches. The equation used to calculate the adjusted values (A) is given below where P is the proportion of total nests attributed to individual females in a year (table 3.3.8.) and C is the mean number of clutches laid by females in the same year (table 3.3.4).

$$A = \frac{1}{P} \times C$$

This value is referred to as the 'adjusted' value for the mean number of clutches laid and is used in calculating population estimate 4 below.

In order to estimate the population of adult females nesting on the Alagadi beach each year, five different methods were used and compared. The data manipulation needed for each method of estimation are given in the results section for ease of interpretation.

Population estimate 1- dividing the total number of nests by a factor of 3, the value commonly taken as the mean number of nests laid by an individual female in a given year (Demetropoulos & Hadjichristophorou 1989; Groombridge 1988; Groombridge 1990; Groombridge & Whitmore 1989).

Population estimate 2 - dividing the total number of nests by the mean number of clutches laid per female (table 3.3.4.).

Population estimate 3 - as estimate 2 using the estimated value for the mean number of clutches laid by individual females (table 3.3.6.).

Population estimate 4 - as estimate 2 but using the adjusted value for the mean number of clutches per female (table 3.3.9.).

Population estimate 5 - the number of known, tagged, individual females nesting each year (tables 3.3.2. & 3.3.3.).

3.3. Results

3.3.1. Nesting activities.

The numbers of nests laid on Alagadi beach, by both species, in each of the years 1992-1996 are illustrated in figure 3.3.1. and given together with the false crawl attempts(FCA) and false crawl U-turns (FCU) in table 3.3.1. Data from 1992 and 1996 are included, courtesy of the Glasgow University Turtle Conservation Project, to illustrate the inter-seasonal variation in the number of activities over a longer time span. It should be noted that in 1992 data recording did not start at this site until July and thus true levels of nesting would have been higher than presented here. In 1992, false crawls were not separated into attempts and U-turns and are given in brackets.

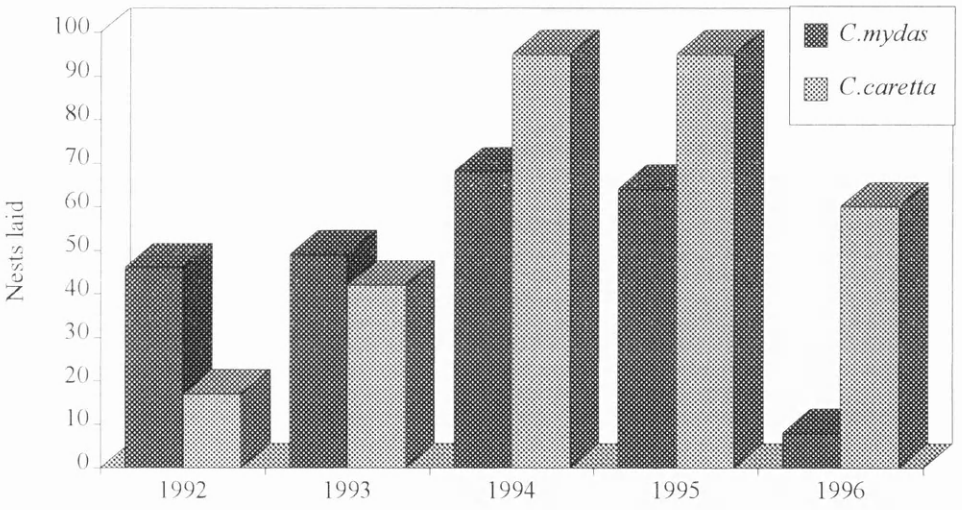


Figure 3.3.1. The number of *C.mydas* and *C.caretta* nests recorded as laid on Alagadi beach in each of the years 1992-1996.

	Nests		FCA		FCU	
Year	***C.mydas***	***C.caretta***	***C.mydas***	***C.caretta***	***C.mydas***	***C.caretta***
1992	46	17	(65)	(33)	-	-
1993	49	42	50	23	37	26
1994	68	95	78	76	52	35
1995	64	95	68	75	83	47
1996	8	60	13	84	7	36

Table 3.3.1. A summary of the activities (nests, false crawl attempts and false crawl U-turns) recorded on Alagadi beach 1992-1996.

3.3.2. Remigration and Fidelity.

Tables 3.3.2. and 3.3.3. show the number of females tagged since the programme was initiated in 1992. The number of females returning in each of the later years and the total number of tagged females ('new' females and remigrants) nesting on the beach in one season are also given. To date 60 *C.mydas* and 99 *C.caretta* have been tagged on Alagadi beach.

	1992	1993	1994	1995	1996	Total
1992	**12**					**12**
1993	0	**16**				**16**
1994	4	0	**18**			**22**
1995	6	0	0	**13**		**19**
1996	0	2	0	0	**1**	**3**

Table 3.3.2. Number of *C.mydas* females tagged in 1992-1996 (in bold) with the number returning in subsequent years. In addition the total number of tagged females nesting on the beach in any one year is shown.

In 1992 and 1993, no *C.mydas* females were remigrants, all being tagged in these years for the first time. In 1994, 18% of nesting *C.mydas* females were remigrants. In 1995 and 1996, these figures had increased to 32% and 67% of the nesting population respectively.

After 2 years, 33% of *C.mydas* females tagged in 1992 had returned and after 3 years this figure had risen to 83%. None of the females tagged in 1992 remigrated after intervals of 1 or 4 years. Of the *C.mydas* females tagged in 1993, none were recorded in 1995 and only 2 (13%) had returned after 3 years. No females tagged in 1994 nested in 1996. No *C.mydas* females have ever returned to nest after an interval of one year. Neither have any *C.mydas* females been recorded nesting in more than two nesting seasons in the five years (1992-1995) of this programme. To date, the mean re-migration interval for *C.mydas* females has been 2.67 years (± 0.14, n=12. ± values given in brackets throughout this thesis are standard errors of the mean). However, care must be taken when quoting this value as not all females are, as yet, accounted for.

	1992	1993	1994	1995	1996	Total
1992	**6**					**6**
1993	0	**9**				**9**
1994	1	1	**30**			**32**
1995	3	(1)	0	**39**		**43**
1996	0 (1)	2 (1)	4	1	**15**	**24**

Table 3.3.3. Number of *C.caretta* females tagged in 1992-1996 with the number returning in subsequent years. In addition the total number of tagged females nesting on the beach in any one year is shown. Figures in brackets are females returning for the second time, since initial tagging year.

Similarly, in 1992 and 1993, none of the *C.caretta* nesting at Alagadi had previously been tagged. In 1994, 6% of nesting females were re-migrants. In 1995 and 1996 remigrants accounted for 11% and 38% of the total population.

Of those tagged in 1992, 17% returned in 1994, and by 1995, this figure had risen to 67%. One *C.caretta*, originally tagged in 1992 was observed to nest in both 1994 and 1996. One female tagged initially in 1993 was recorded as nesting in each subsequent year, laying one clutch in 1993, 1994 and 1995 and 3 clutches in 1996. Of the females tagged in 1993, 33% had returned by 1996. 13% of females tagged in 1994 nested in 1996 and 3% tagged in 1995 returned in 1996. In 1995, one *C.caretta* female tagged in 1992 was observed but did not successfully nest. This turtle is not included in the data presented in table 3.3.3.

To date, the mean re-migration interval for *C.caretta* females has been 2.07 years (± 0.21, n=15), although *C.caretta* females appear to equally favour intervals of 1, 2 and 3 years (n=4,6,5 respectively). Again many females are still unaccounted for and only when the maximum re-migration interval is known can these values be calculated accurately.

In 1996, night time work was also carried out by a group on some neighbouring beaches. During this study one *C.mydas* and one *C.caretta* nesting and tagged at Tatlisu beach (~ 40 kilometres east) were also recorded at Alagadi. However neither successfully nested at the latter site. In addition one *C.caretta* tagged at Alagadi in 1994 was recorded nesting on a beach over 100 km east in 1996, but was not observed at Alagadi in that year.

3.3.3. The number of clutches laid.

The number of individuals observed laying 1-5 clutches in a season is given in table 3.3.4. and illustrates the fact that, in all years, *C.mydas* females laid a greater number of clutches than *C.caretta.* Collectively, over the five years, the mean number of clutches laid by *C.mydas* females was 2.25 (± 0.14, n=72) and for *C.caretta* 1.54 (± 0.08, n=114).

The number of clutches laid by females returning after intervals of 1-4 years is given in table 3.3.5. The largest number of clutches were laid by *C.mydas* females returning after a three year interval and *C.caretta* females returning after two years. For both species a

greater number of clutches are laid by remigrants in these peak years than by the overall population.

	Number of clutches recorded in a season					
Year	1	2	3	4	5	Mean
C.mydas 1992	5	4	3	0	0	**1.83**
1993	7	4	3	2	0	**2**
1994	10	5	5	2	0	**1.96**
1995	4	2	3	8	2	**3.12**
1996	1	1	1	0	0	**2**
C.caretta 1992	6	0	0	0	0	**1**
1993	7	2	0	0	0	**1.2**
1994	20	10	2	0	0	**1.44**
1995	29	6	6	2	0	**1.56**
1996	12	6	4	1	1	**1.88**

Table 3.3.4. The number of females each year observed to lay 1-5 clutches in the season.

		Number of clutches recorded in a season					
Remigration (years)		1	2	3	4	5	Mean
C.mydas	1	0	0	0	0	0	**0**
	2	0	1	3	0	0	**2.75**
	3	0	2	2	2	2	**3.5**
	4	0	0	0	0	0	**0**
C.caretta	1	3	0	1	0	0	**1.5**
	2	0	2	2	1	1	**3.17**
	3	2	1	2	0	0	**2**
	4	0	0	0	0	0	**0**

Table 3.3.5. The number of observed clutches laid by females returning after 1-4 years.

In order to gain a more accurate estimate of the number of clutches a female laid in a season, the inter-nesting intervals were examined (as per Addison 1996). Figures 3.3.2. and 3.3.3. illustrate the recorded frequency of intervals between clutches. The tri-modal nature of these histograms strongly suggests that the peaks seen after 20 days are the result of previous nests of the individual going unrecorded.

When the inter-nesting interval was more than 18 days and fell into the second peak, it was assumed that females had laid another clutch and when greater than 36 days, in the third peak, had laid two extra clutches. The data were altered accordingly and the new estimated values are given below in tables 3.3.6. and 3.3.7.

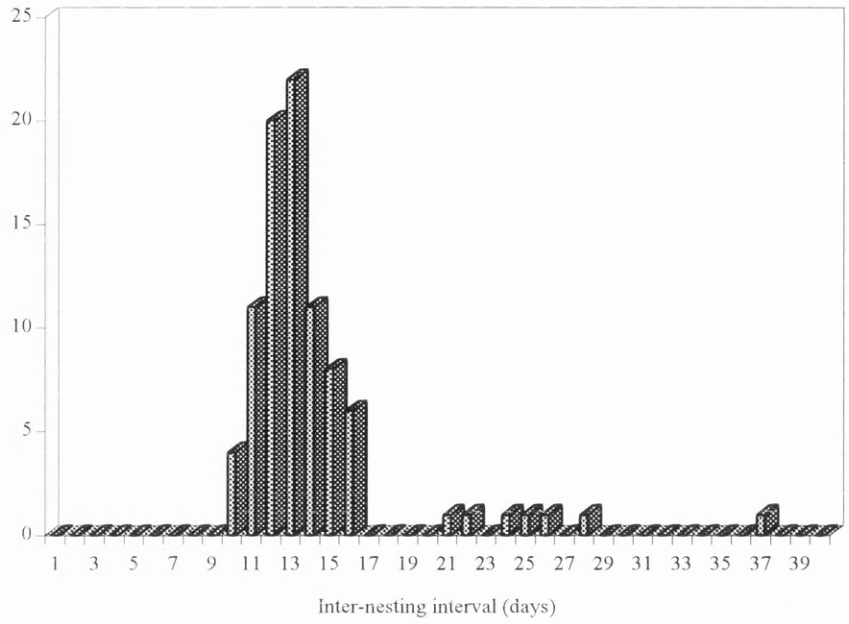


Figure 3.3.2. Frequency histogram of inter-nesting intervals recorded in *C.mydas* females.

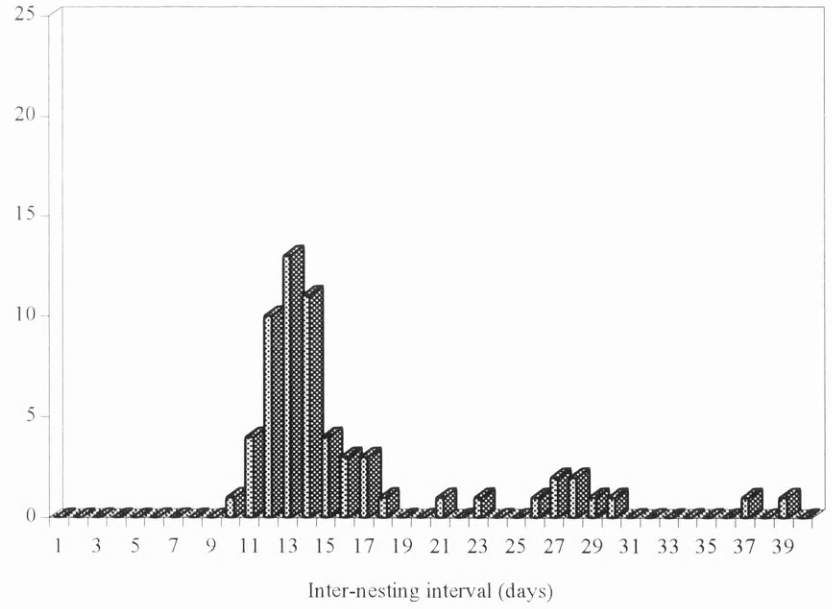


Figure 3.3.3. Frequency histogram of inter-nesting intervals recorded in *C.caretta* females.

The overall mean number of clutches calculated using this method was 2.35 (± 0.15, n=72) for *C.mydas* females, and 1.63 (± 0.09, n=114) for *C.caretta*. *C.mydas* remigrants laid on average 3.58 clutches (±0.26, n=12) and *C.caretta* 2.53 clutches (±0.38, n=15). The figures in brackets represent the percentage of nests laid which are now accounted for. Thus it is likely that the values obtained in 1995 for *C.mydas* (3.26) and 1996 for *C.caretta* (2) give the most accurate estimate of the mean number of clutches laid for these species respectively.

	Estimated number of clutches laid in a season					
Year	**1**	**2**	**3**	**4**	**5**	**Mean**
***C.mydas* 1992**	5	3	4	0	0	**1.92**
1993	7	4	2	3	0	**2.06 (69 %)**
1994	11	3	4	4	0	**2.05 (66%)**
1995	4	1	3	8	3	**3.26 (99%)**
1996	1	1	1	0	0	**2 (75%)**
***C.caretta* 1992**	6	0	0	0	0	**1**
1993	7	0	1	1	0	**1.56 (34%)**
1994	20	9	2	1	0	**1.5 (57%)**
1995	29	6	3	5	0	**1.63 (75%)**
1996	12	5	4	1	2	**2 (82%)**

Table 3.3.6. Estimated values for the number of females each year to lay 1,2,3,4 or 5 clutches in the season. The percentage of the total number of nests accounted for by these values is given in brackets.

		Estimated number of clutches laid in a season					
Remigration (years)		**1**	**2**	**3**	**4**	**5**	**Mean**
C.mydas	**1**	0	0	0	0	0	**0**
	2	0	0	3	1	0	**3.25**
	3	0	1	2	3	2	**3.75**
	4	0	0	0	0	0	**0**
C.caretta	**1**	3	0	1	0	0	**1.5**
	2	0	2	1	1	2	**3.5**
	3	2	1	1	1	0	**2.2**
	4	0	0	0	0	0	**0**

Table 3.3.7. Estimated values for the number of clutches laid by females returning after an interval of 1-4 years.

In the examination of the individual variation that exists in inter-nesting intervals, no significant difference was recorded in *C.caretta* females (F=2.06, p=0.147, n=31), however sample sizes were small and it was rare to record more than two inter-nesting intervals for the same *C.caretta* female. In the case of *C.mydas* a significant result was recorded

(F=4.12, p<0.001, n=53) and the intra class correlation coefficient was r=0.46. This means that the inter-nesting intervals of individual females have a moderate (Martin & Bateson 1986) repeatability, indicating that within females, variation in their inter-nesting intervals is not high. It is therefore very unlikely that a female will have an inter-nesting interval of 10 days followed by one of 20 days.

In order to gauge the reliability of observations, table 3.3.8. illustrates the percentage of the total number of nests recorded that were i) observed and ii) attributed to known females. When estimating female reproductive output and population size this information should be considered in the light of a possible underestimate of the number of clutches a female lays. These data illustrate the higher proportion of *C.caretta* activities that were not actually witnessed, and also indicates how, over the years, a greater proportion of activities were observed for both species.

	Chelonia mydas		*Caretta caretta*	
Year	**Observed**	**Attributed**	**Observed**	**Attributed**
1993	73 %	67 %	48 %	26 %
1994	71 %	63 %	53 %	40 %
1995	97 %	95 %	74 %	72 %
1996	100 %	75 %	78 %	77 %

Table 3.3.8. The percentage of the total number of nests recorded on Alagadi beach 1993-1996 that were i) observed and ii) attributed to known females.

By taking into account the proportion of activities that were observed and attributed to individual females, new values for the mean number of clutches laid can be calculated and are given in table 3.3.9. The equation used to calculate the adjusted values is given in section 3.2. These values account for 100% of the total number of nests recorded.

Year	*C.mydas*	*C.caretta*
1993	2.99	4.61
1994	3.11	3.6
1995	3.28	2.17
1996	2.66	2.44

Table 3.3.9. Adjusted values for mean number of clutches laid per female for *C.mydas* and *C.caretta*, 1993-1996.

3.3.4. Tag loss

In this study females were tagged on each foreflipper to reduce the effect and allow quantification of tag loss. After an interval of 2 years 12.5% of tags on *C.mydas* females had been lost (one of eight tags). After 3 years 20 % of tags were missing

(three of fifteen tags). In all cases these females were returning after nesting initially in 1992 and 1993 and had been tagged with 'Jumbotags'. Collectively this gives a mean tag loss of 6.5 % per annum for the 'Jumbotag' on *C.mydas* turtles. No *C.mydas* females tagged with 'Supertags' (initiated in 1994) have returned to nest and hence their success cannot be evaluated in this species.

In the case of *C.caretta* females, and with respect to the 'Jumbotags', after 2 years 50% tag loss had occurred (one of two tags), after 3 years 8 % (one of thirteen tags) had been lost and after 4 years one female still had both of her tags intact. Collectively this gives a mean tag loss of 6.9% per annum. Of the 'Supertags' one female returned after 1 year and had lost neither tag. After 2 years 50% of 'Supertags' had been lost (four of eight tags). Sample sizes are too small, at present, to analyse whether the 'Supertag' is lost more than the 'Jumbotag'. A degree of wear was also seen in the 'Supertag' with the numbers becoming difficult to read. Any tags which were lost, faded or loose were replaced or an additional tag attached.

3.3.5. Population estimates.

The results of the five methods of estimating the population of marine turtles nesting on Alagadi are given below in tables 3.3.10. and 3.3.11. for *C.mydas* and *C.caretta* respectively.

Year	Population estimate 1	Population estimate 2	Population estimate 3	Population estimate 4	Population estimate 5
1992	15	25	24	-	12
1993	17	25	24	15	16
1994	23	35	33	22	22
1995	22	21	20	20	19
1996	3	4	4	3	3

Table 3.3.10. Resultant population estimates from five different methods described in section 3.2 for *C.mydas*.

Year	Population estimate 1	Population estimate 2	Population estimate 3	Population estimate 4	Population estimate 5
1992	6	17	17	-	6
1993	14	35	27	9	9
1994	32	66	63	26	32
1995	32	61	58	44	43
1996	20	32	30	25	24

Table 3.3.11. Resultant population estimates from five different methods described in section 3.2 for *C.caretta*.

3.4. Discussion

In 1992, a pilot study was undertaken by the Glasgow University Turtle Conservation Project, identifying Alagadi as the main nesting site for marine turtles in the north of Cyprus. In that year data collection was not complete with surveying and tagging only starting in July. Each subsequent season, daily monitoring of the Alagadi beach has taken place throughout the nesting season, with all activities being recorded. For this reason, although data from 1992 are presented in parts, complete records are not held. Therefore, only data from 1993-1996 should be considered when assessing true estimates of numbers of clutches laid and population sizes.

1996 witnessed a dramatic decline in the number of *C.mydas* females nesting at Alagadi. In that year, however, fewer *C.mydas* were also recorded on the 30 beaches around Northern Cyprus, suggesting that the phenomenon was not site specific (Godley & Kelly 1996). Studies of marine turtle nesting populations report that annual fluctuations in *C.mydas* nesting are more commonly recorded than in *C.caretta* populations. Years with very low nesting numbers are frequently followed by exceptionally prolific years (Bagley *et al.* 1996; Ehrhart *et al.* 1996; Limpus 1996b).

It must however be stressed that the results presented in this thesis on remigration intervals and clutch frequency are not definitive and females may be nesting elsewhere both within and between nesting seasons. Until sophisticated tracking equipment can be used to clarify these possibilities results must be treated with caution.

Of the *C.mydas* females tagged in 1992, 33% returned after a 2 year interval whilst 50% returned after 3 years. Of those tagged in 1993, 13% returned in 1996. The time scale of this thesis is too limited to make generalisations, however these data may indicate emerging patterns of preferred remigration intervals of 2 or 3 years in this species.

From this study, *C.caretta* females appear to equally favour remigration intervals of 1, 2 and 3 years. Unlike *C.mydas* females they are able to return to nest after only a one year interval. Environmental factors such as temperature and food quality and quantity during both the inter-nesting and inter-season period will be important in determining at what frequency a female nests. Certainly the fact that no *C.mydas* females have been

recorded nesting after an interval of one year suggests that this 12 month period is not long enough to lay down enough reserves in preparation for the larger number of clutches laid by this species. It may also be the distance to the over-wintering grounds that determines how frequently a female nests, as migration will also use up reserves (Limpus 1997). In the Mediterranean it is not known where females go between nesting seasons and how far they must therefore migrate for each breeding attempt.

This tagging programme is still in its early stages and longer remigration intervals may be recorded in future years, with females returning after intervals of more than four years. The remigration pattern will always remain an estimated value since mortality, tag loss and emigration will all affect the values obtained. Certainly there is some evidence that the latter may be the case as one *C.caretta* female tagged at Alagadi in 1994 was recorded nesting elsewhere in 1996, and several have been recorded at more than one site within a season.

Many studies examining site fidelity of nesting turtles have suggested that females return to areas of coastline as opposed to specific beaches (Johnson & Ehrhart 1994; Tarlbert *et al.* 1980). It is feasible that those studies reporting high fidelity to beaches are conducted in very isolated areas with no other suitable beaches in the vicinity. As Hays and Sutherland (1991) suggest, a lack of fidelity allows colonisation of other nesting sites. This is crucial if a particular nesting beach becomes unsuitable for nesting. Over the decades, beaches change naturally due to the forces of erosion and may, for example, become too stony for nesting. Similarly beach development may render a beach unsuitable. High site fidelity may thus be detrimental to the species if they are unable to adapt to changes at a particular location.

There is a possible link between the remigration intervals of females and the number of clutches they laid; thus *C.mydas* females returning after an interval of 3 years laid, on average, more clutches than those returning after 2 years. It is of interest to note that females following this pattern laid fewer clutches in their initial year of tagging. *C.caretta* females displayed the same phenomenon with more clutches being deposited after a 2 year interval. Thus it appears that the number of clutches a female lays in a season may be affected by both the length of the remigration interval and reproductive output in the previous season.

The data suggests that remigrating females lay a greater number of clutches than the average. Whilst this may be in some way related to the size or age of the turtle it might be suggested that as these females had been tagged in previous years there was a greater chance of them being recorded returning to nest. However, the high proportion of activities that are attributed to an individual female indicate that this is unlikely to be the case.

The average number of clutches recorded for females in each year of this study is a minimum, as some nests undoubtedly will not have been observed. For this reason data were manipulated in order to gain a more accurate estimate of the mean number of clutches laid by females in a given year. Whilst estimate 3 takes into account some of these 'missed nestings' by examining inter-nesting intervals, where a female only nested once no correction was possible. Thus estimate 3 will tend to produce a lower estimate of clutch numbers. Estimate 4 on the other hand takes into account all nests laid on the beach and adjusts the mean number of clutches accordingly, but it can only be accurate if all females nesting on a beach are tagged. As a result it is likely to provide an over estimate of the number of clutches laid per female.

It is known that at least 60 *C.mydas* and 99 *C.caretta* females have nested at Alagadi in the past five nesting seasons, this being the total number of females tagged since 1992. Whilst this is the absolute minimum number of females, it is possible to attempt to gain a more accurate value of the population from the data recorded or adjusted in this chapter.

Many studies estimate that marine turtles lay on average 3 clutches per season. This method was used by Groombridge (1990) to calculate the overall population in the Mediterranean and was used in this chapter to give population estimate 1. Although the current findings have shown that *C.mydas* females do indeed lay in the region of three clutches per season, with *C.caretta* this method is likely to lead to an under-estimate as the findings of the current study indicate that *C.caretta* females lay less than 3 clutches, on average, per annum in Cyprus. If this is also the case in other areas of the Mediterranean there may be up to a third more *C.caretta* females nesting annually than the estimated 2000 (Groombridge 1990). The values obtained for method 2 are likewise spurious because they only account for the minimum number of clutches laid by females; i.e. those that were observed. Using a combination of estimates 3, 4 and 5, it is

possible to derive fairly accurate maximum and minimum population numbers. The latter methods incorporate inter-nesting intervals, mean clutch numbers and tagged females, and so are more inclusive in their approach. Estimate 3 provides the maximum value and the greater of estimates 4 or 5, the minimum. The population ranges for both species in each of the years of this study are given in table 3.4.1.

	Chelonia mydas	*Caretta caretta*
1992	12-24	6-17
1993	16-24	9-27
1994	22-33	32-63
1995	20-20	44-58
1996	3-4	25-30

Table 3.4.1. The ranges in population estimates of *C.mydas* and *C.caretta* nesting at Alagadi beach.

For both species however, Alagadi beach has been shown to host a small but nevertheless significant number of nests each year. Excluding 1996, Alagadi supported in the region of 15-18% of the *C.mydas* and 17-19% of the *C.caretta* nests recorded in Northern Cyprus (Broderick & Godley 1993; Broderick & Godley 1995; Godley & Broderick 1994). Taking into consideration that there may be as few as 300-500 *C.mydas* nesting females left in the Mediterranean (Groombridge 1990), the importance of Alagadi and Northern Cyprus collectively for this species is apparent.

Chapter 4 - Nesting Ecology of *Chelonia mydas* and *Caretta caretta*, at Alagadi.

4.1. Introduction

Although the Mediterranean supports a population of *C.mydas*, as discussed in chapter 3, numbers are low and as a consequence little available information exists regarding the nesting ecology of the species in this area. Where studies have been published, data sets are, as a consequence, small. The data presented in this chapter, and indeed the remainder of this thesis, are therefore an important contribution to the understanding of the biology of *C.mydas* nesting in these waters.

As a source of reference, published data on nesting and hatching parameters from other Mediterranean studies are presented in tables 4.1.1. and 4.2.2. for both *C.mydas* and *C.caretta* respectively. Information is also presented from a general, world-wide, review for each species (Dodd 1988; Marquez 1990).

The mean inter-nesting period for *C.mydas* world-wide is estimated as between 10 and 14 days (Miller 1996). No relevant data have been published for the mean inter-nesting periods of *C.mydas* nesting in the Mediterranean. Margaritoulis (1989) quotes the inter-nesting period for *C.caretta* in Greece as 15.2 days, which is well within the global range of 12-17 days (Dodd 1988).

C.mydas females, nesting in the Mediterranean, have a mean curved carapace length of 90-96 cm, somewhat smaller than those found in other parts of the globe but well within the range for the species of 81-112 cm (Baran & Kasparek 1989a; Coley & Smart 1992; Gerosa *et al.* 1995; Marquez 1990). All studies of *C.caretta* in the Mediterranean have shown them to be smaller in size than those found nesting outwith (Baran & Kasparek 1989a; Dodd 1988; Erk'akan 1993; Margaritoulis 1989; Sutherland 1985). Additionally, within the Mediterranean, variation is seen in the size of nesting females. *C.caretta* nesting in Turkey appear to be smaller than those found in Greece (Baran & Kasparek 1989a; Erk'akan 1993; Margaritoulis 1989; Sutherland 1985).

Incubation periods are reduced where higher temperatures prevail (Mrosovsky *et al.* 1995). For *C.mydas* nesting in Turkey, a mean incubation period of 53.8 days has been reported at Akyatan, one of the most southerly nesting sites in Turkey (Gerosa *et al.* 1995). This is the only available information for Mediterranean *C.mydas,* and is within the range of 48-70 days found world-wide (Marquez 1990). A range of mean incubation periods, from 54.8-

59.3 days, are presented in table 4.3.2. for *C.caretta* in Turkey (Erk'akan 1993; Peters & Verhoeven 1992) and Greece (Margaritoulis 1989; Sutherland 1985). These all fall within the world-wide values (Dodd 1988). From the data published, there does not appear to be a difference between the length of time that nests incubate in Greece and Turkey.

The mean clutch sizes laid by *C.mydas* females nesting in Turkey range between 100-123 eggs (Gerosa *et al.* 1995; Peters & Verhoeven 1992), and accord well with the findings of Marquez (1990) for the species globally. Whilst the mean clutch sizes of *C.caretta* nesting in Greece are within the world-wide range, those recorded in Turkey, are smaller than those found elsewhere (Dodd 1988; Erk'akan 1993; Margaritoulis 1989; Margaritoulis *et al.* 1995; Peters & Verhoeven 1992; Sutherland 1985).

In the literature surveys, only one record was found of a hatching success of 67% of the eggs of *C.mydas* nests in the Mediterranean. This was at the Göksu Delta, Turkey (Peters & Verhoeven 1992), however the sample size was very small, (seven nests). The hatching success of *C.caretta* nests varies widely at different sites around the Mediterranean, although all are within the ranges found world-wide (Margaritoulis & Dimopoulos 1995; Margaritoulis *et al.* 1995; Peters & Verhoeven 1992).

No data on hatchling emergence success or hatchling carapace measurements are available for *C.mydas* in the Mediterranean. In the case of *C.caretta*, data are available although variation in methodology makes comparisons unreliable (Margaritoulis & Dimopoulos 1995; Margaritoulis *et al.* 1995; Peters & Verhoeven 1992; Sutherland 1985).

There is much contradictory evidence as to whether variables such as clutch size and hatching success vary within the season. Some studies have shown increases in hatching success as the season progressed whilst others have recorded a decrease. Often relationships are available at the population level and not at the individual level (Bjorndal & Carr 1989; Davis & Whiting 1977; Frazer and Richardson 1986; LeBuff & Beatty 1971).

In this chapter, nesting and hatching data are presented for both *C.mydas* and *C.caretta* females nesting at Alagadi in the 1993-1995 seasons. Variations between the three years of this study are analysed and where possible statistical comparison made to other nesting sites in the Mediterranean. In addition, the variation that exists in the measured parameters was examined in relation to the number of clutches a female laid.

Locality	*Reference*	*Carapace length (cm)*	*Inter-nesting period (days)*	*Incubation period (days)*	*Clutch size*	*Hatching success (%)*	*Hatchling Emergence success (%)*	*Hatchling length (cm)*
General, Turkey	Baran & Kasparek 1989a	90.1 ± 0.81, n=42 (C)	----	----	----	----	----	----
Akyatan, Turkey	Gerosa *et al.* 1995	92.1 ± 1.05, n=13 (C)	----	53.8 ± 0.94, n=23	122.9	----	----	----
Göksu Delta, Turkey	Peters & Verhoeven 1992	----	----	----	101.1 ± 8.7, n=9	68.7 ± 9.88, n=7	----	----
Kazanli, Turkey	Coley & Smart 1992	96 ± 4, n=4 (C)			122 ± 17, n=7			
World-wide	Marquez 1990	81-112 (C)		48-70	85-144			

Table 4.1.1. A review of *Chelonia mydas* mean nesting and hatching parameters recorded in the Mediterranean, with standard errors and sample sizes where this information was available.

Key

C-curved carapace length measurement

Locality	*Reference*	*Carapace length (cm)*	*Inter-nesting period (days)*	*Incubation period (days)*	*Clutch size*	*Hatching success (%)*	*Hatchling Emergence success (%)*	*Hatchling length (cm)*
Dalyan, Turkey	Erk'akan 1993	73.1 ± 0.75, n=49 (N)	----	59.3 ± 0.57, n=47	73.4 ± 1.39, n=235	----	----	----
Göksu Delta, Turkey	van Piggelen 1993	----	----	56.96 ± 1.19, n=25	----	----	----	----
General, Turkey	Baran & Kasparek 1989a	75.6 ± 0.6, n=81 (C)	----	----	----	----	----	----
Göksu Delta, Turkey	Peters & Verhoeven 1992	----	----	54.8 ± 0.62, n=33	91.7 ± 7.35, n=44	77 ± 4.59, n=33	63 ± 4.95, n=33	3.91 ± 0.27, n=37*
Zakynthos, Greece	Sutherland 1985	81.2 ± 0.06, n=95 (N)	----	57.8 ± 2.8, n=6	114 ± 1.03, n=52	----	----	4.0 ± 0.011, n=221
Kiparissia Bay, Peloponnesus, Greece	Margaritoulis 1989	83.1 ± 0.56, n=72 (C)	15.2 ± 0.56, n=72	55.5 ± 0.89, n=50	117 ± 3.18, n=52	----	----	----
Lakonikos Bay, Peloponnesus, Greece	Margaritoulis *et al.* 1995	----	----	----	113.1, n=174	74.8, n=174	71, n=174	----
Zakynthos, Greece	Margaritoulis & Dimopoulos 1995	----	----	----	----	67.7, n=572	60.1, n=572	----
World-wide	Dodd 1988	84.7-105.1 cm (C)	12-17	50.2-69	94.6-149	53.1-83.4	----	----

Table 4.1.2. A review of *Caretta caretta* mean nesting and hatching parameters recorded in the Mediterranean, with standard errors and sample sizes where this information was available.

Key C-curved carapace length measurement;

N-method not recorded;

*-In the case of Peters & Verhoeven (1992) hatchling size was taken from hatchlings found on the beach;

#-In the case of Margaritoulis & Dimopoulos (1995) live hatchlings found in the nest were classified as having failed to emerge from the nest.

4.2. Methodology

The protocol followed for conducting beach surveys is given in chapter 2. Some data manipulation was necessary before statistical analyses could be conducted. Where a female nested more than once in a season, and variation existed in the curved carapace measurements recorded, her mean length and width were calculated for use in analyses. Similarly, where a female nested more than twice, her mean inter-nesting period was calculated. One female nested in more than one of the years of this study and was removed from the data set. This was to avoid the problem of pseudoreplication (Hurlbert 1984).

For hatching parameters such as incubation period, clutch size, hatching success, emergence success and hatchling size, no correction was made. All recorded values were entered into the data set independently. Hatchlings which were discovered alive in the sand were measured using callipers, to record their straight carapace length and width. The mean straight carapace lengths and widths of individuals in any one nest were calculated so that only one measurement was entered into the data set for that clutch. Again, this ensures that the offspring of one female are not over represented in the data set.

As in chapter 3, where an inter-nesting interval of greater than 18 days was recorded it was assumed that the female had laid a clutch since she was last recorded nesting. For the examination of variables according to clutch order it was necessary to correct the data according to this method.

Statistical analyses were carried out to compare variation in parameters with respect to the three years of this study. This was carried out using a one way ANOVA (followed by a Scheffé test where a significant result was found) or a t-test depending on the number of years in the comparison. If the data were non-parametric a Kruskal-Wallis test was used. For some variables, data collected over the three years were pooled to gain an overall mean for Cyprus. This was to enable a comparison of the results obtained with those recorded elsewhere in the Mediterranean. Data presented in tables 4.1.1. and 4.1.2. were compared statistically with overall means from Cyprus by way of a t-test. In addition, the variation that exists with respect to clutch order was examined using a one way ANOVA. For each analysis, residuals were tested for normality. If data were not normally distributed, they were transformed to increase normality or analysed using a non parametric test.

4.3. Results

The means, with standard errors and sample sizes of nesting and hatching parameters recorded in this study are given in table 4.3.1 for *C.mydas* and 4.3.2 for *C.caretta*.

4.3.1. Nesting parameters

Inter-nesting periods for *C.mydas* ranged from 10-16 days, whereas inter-nesting intervals for *C.caretta* were slightly longer, ranging from 10-18 days. There were no significant differences between the inter-nesting periods recorded for *C.mydas* ($F=0.27$, $n=33$, $p=0.764$) in the three years or for *C.caretta* females in 1994 and 1995 ($t=1.03$, $n=24$, $p=0.32$).

The annual mean curved carapace length of nesting *C.mydas* females ranged from 88.9 to 95.6 cm (absolute range 78.7-105.7 cm) and annual mean widths ranged from 80.7 to 84.8 cm (absolute range 63.0-96.7 cm). The variation in measurements of curved carapace length and width, with respect to any one year, were examined using a one way ANOVA. It was found that there was a significant difference between the curved carapace lengths of *C.mydas* females ($F=9.31$, $p=0.0003$, $n=57$). The Scheffé test showed that this difference could be attributed to the fact that females nesting in 1994 were significantly larger than those in 1993 and 1995. The same difference was found when examining the curved carapace width of females ($F=4.08$, $p=0.023$, $n=57$).

The range of annual mean curved carapace lengths of the smaller *C.caretta* was between 72.5 and 77.9 cm (absolute range 65.0-86.5 cm) and annual mean widths ranged from 64.5 to 68.2 cm (absolute range 54.5-77 cm). A significant difference was found with respect to the curved carapace lengths of *C.caretta* females nesting yearly. Females nesting in 1993 were significantly larger than those in 1994, but not 1995 ($F=3.40$, $p=0.039$, $n=72$). No significant difference was found in the sizes of the curved carapace widths ($F=1.04$, $p=0.361$, $n=72$).

A graphical comparison of the curved carapace lengths of both species for each of the three years is given in figure 4.3.1.

Parameter	1993			1994			1995		
	Mean	*s.e.*	*n*	*Mean*	*s.e.*	*n*	*Mean*	*s.e.*	*n*
Inter-nesting period (days)	13.2	0.61	9	13.12	0.38	10	12.79	0.36	14
Adult size (cm)									
Curved carapace length	88.9	1.21	16	95.6	1.03	22	90.5	1.44	19
Curved carapace width	80.9	1.24	16	84.8	1.5	22	80.7	1.76	19
Incubation period (days)	50.6	0.57	24	51.4	0.53	45	51.1	0.55	52
Clutch size	103.4	2.58	26	123.4	5.48	53	107.3	3.97	51
Hatching success (%)	85.5	2.6	26	87.7	2.4	53	86.1	2.3	51
Hatchling emergence success (%)	86.4	2.6	26	81.9	2.3	53	84.9	2.3	51
Depth									
Top (cm)	61.2	3.21	24	70.7	1.63	48	67.5	1.39	50
Bottom (cm)	78.4	2.31	24	87.2	2.07	48	85.9	1.78	50
Hatchling size (cm)									
Straight carapace length	4.5	0.06	13	4.7	0.03	36	4.6	0.04	29
Straight carapace width	3.5	0.05	13	3.5	0.03	36	3.5	0.04	29

Table 4.3.1. Mean nesting and hatching parameters of *C.mydas* nesting at Alagadi for each of the three study years together with standard errors and sample sizes.

Parameter	1993			1994			1995		
	Mean	*s.e.*	*n*	*Mean*	*s.e.*	*n*	*Mean*	*s.e.*	*n*
Inter-nesting period (days)	-	-	-	13.82	0.52	11	13.13	0.42	13
Adult size (cm)									
Curved carapace length	77.9	1.83	8	72.5	0.77	25	73.3	0.78	39
Curved carapace width	68.2	1.9	8	64.5	0.73	25	65.5	0.75	39
Incubation period (days)	47.9	0.62	17	47.9	0.36	58	48.1	0.55	40
Clutch size	79.3	4.5	22	65.7	2.5	62	68.7	3.3	27
Hatching success (%)	78	3.0	22	84.3	2.8	61	68.8	4.6	26
Hatchling emergence success (%)	80.8	2.9	22	75.8	2.2	61	68.4	4.3	26
Depth									
Top (cm)	38.4	1.81	20	38.2	0.85	59	39.6	1.65	28
Bottom (cm)	53.7	1.96	20	49.7	0.92	59	53	1.52	28
Hatchling size (cm)									
Straight carapace length	4.08	0.05	15	4.07	0.02	49	3.95	0.05	19
Straight carapace width	3.21	0.08	15	3.07	0.03	49	3.05	0.07	19

Table 4.3.2. Mean nesting and hatching parameters of *C.caretta* nesting at Alagadi for each of the three study years together with standard errors and sample sizes.

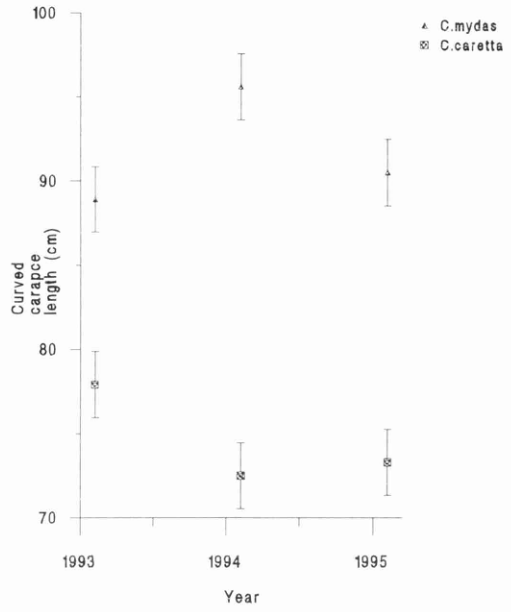


Figure 4.3.1. The mean curved carapace lengths of *C.mydas* and *C.caretta* females nesting at Alagadi in the years 1993-1995 with 95% confidence limits.

4.3.2. Hatching parameters.

Incubation periods were longer in *C.mydas*, with annual means ranging from 50.6-51.4 days, (absolute range 44 -59 days) compared to *C.caretta* nests which had mean incubation periods ranging from 47.9-48.1 days, (absolute range 42-60 days). No significant inter-annual differences were recorded between the incubation periods of either *C.mydas* (F=0.434, p=0.65, n=121) or *C.caretta* (F=0.476, p=0.934, n=115) nests.

Yearly mean clutch sizes of *C.mydas* ranged from 103.4 to 123.4 eggs (range 23-199 eggs) with *C.caretta* mean clutch sizes ranging from 65.7 to 79.3 eggs (range 9-115). The clutch sizes recorded in 1994 were found to be significantly larger than those laid in 1993 and 1995 for *C.mydas* (F=3.94, p=0.022, n=130). *C.caretta* clutches laid in 1993 were significantly larger than those laid in 1994 but not 1995 (F=3.58, p=0.031, n=111). Whilst significant annual variation was recorded in the clutch sizes when examining the raw data this was not found to be the case when examining either mean clutch (*C.mydas* F=3.19, p=0.052, n=48; *C.caretta* F=1.87, p=0.167, n=45) size of nesting females or one randomly selected nest for each individual female (*C.mydas* F=1.36, p=0.268, n=48; *C.caretta* F=1.83, p=0.174, n=45).

This is likely to be due to the annual variation similarly recorded in female size and the relationship that exists between the latter parameter and clutch size. This relationship is examined further in chapter 5. However, to clarify this point at this stage a General Linear Model was used to perform an analysis of covariance to examine whether the relationships of female size and clutch size varied over the three years of this study. For both species, whilst there was a relationship between female size and mean clutch size (see chapter 5) there was no significant difference in the slope (*C.mydas* F=0.95, p=0.397, n=48; *C.caretta* F=0.20, p=0.823, n=48) or elevation (*C.mydas* F=0.28, p=0.755, n=48; *C.caretta* F=1.09, p=0.0.346, n=48) with respect to year. Similarly no significant variations in slope (*C.mydas* F=0.45, p=0.641, n=48; *C.caretta* F=0.39, p=0.677, n=48) or elevation of the relationship between female size and clutch size was recorded for either species (*C.mydas* F=0.02, p=0.980, n=48; *C.caretta* F=0.99, p=0.383, n=48). Thus there was therefore no year effect on clutch size for either species.

Of the nests which hatched, percentage success was high for both species. The mean hatching success for *C.mydas* varied between 85.5 and 87.7% (range 5-100%), and for *C.caretta* 68.8 and 85.3% (range 9-99). No significant variations with respect to year were recorded for *C.mydas* (H=0.51, p=0.775, n=130) although such a difference was recorded in the success of *C.caretta* (H=8.52, p=0.014, n=109) nests. Hatching success of *C.caretta* nests in 1993 was significantly higher than in 1995 but not 1994. Emergence success ranges for *C.mydas* were 5-100% and *C.caretta* 9-98%. No significant difference was found in the emergence success of nests in each of the three years for either *C.mydas* (H=3.15, p=0.207, n=130) or *C.caretta* (H=4.55, p=0.104, n=109).

With reference to the actual nests, depths ranged from 21-102 cm to the top of the egg chamber for *C.mydas* and 22-69 cm for *C.caretta*. Depth to the bottom of the egg chamber ranged from 52-135 for *C.mydas* and 36-78 cm for *C.caretta*. *C.mydas* nests were significantly deeper in 1994 than in 1993, with respect to both the top (F=5.23, p=0.007, n=122) and bottom (F=3.85, p=0.024, n=122) of the egg chamber. The depths of both the top (F=0.29, p=0.746, n=107) and bottom (F=2.75, p=0.068, n=107) of the egg chambers of *C.caretta* nests showed no significant differences between the years.

Hatchling lengths ranged from 3.7 to 5.4 cm for *C.mydas* and 2.9 to 4.9 cm for *C.caretta*. Hatchling widths ranged from 2.56-4.96 cm for *C.mydas* and 2.38 to 4.4 cm for *C.caretta*. No significant differences were found between the straight carapace widths of *C.mydas*

hatchlings over the three years (F=0.72, p=0.490, n=77) whereas the straight carapace lengths were significantly different (F=5.17, p=0.008, n=77). Hatchlings in 1994 were significantly longer than those of 1993 and 1995. No significant differences were recorded in the straight carapace lengths (F=2.64, p=0.77, n=82) or widths (F=2.34, p=0.103, n=82) of *C.caretta* hatchlings between years.

4.3.3. Clutch order.

As well as examining the level of annual variation that exists, the variation in the inter-nesting period, clutch size, incubation period, hatching success, hatchling emergence success, depth of the nest and hatchling size with respect to clutch order was also examined. In the case of *C.mydas* clutches 1-5 could be compared. For *C.caretta* however, sample sizes from clutches 1-3 only were available.

For *C.mydas*, no significant differences were recorded, with respect to clutch order, for the inter-nesting periods (F=1.61, p=0.195, n=83), hatchling emergence success(H=5.21, p=0.268, n=95), depth, either the top (F=0.67, p=0.617, n=92) or bottom (F=1.29, p=0.279, n=89) of the nest, or either of the measurements of hatchling size (length; F=0.8, p=0.502, n=61, width; F=0.05, p=0.986, n=61). However a difference was recorded in the incubation periods of the clutches of *C.mydas*. Clutch 3 had a significantly shorter incubation period than clutches 1 and 5 (F=3.14, p=0.029, n=96). This result is illustrated in figure 4.3.2. A significant difference was also recorded, for *C.mydas*, with respect to clutch size, (F=2.76, p=0.032, n=91) with clutch one being significantly smaller than clutch 3. This result is illustrated in figure 4.3.3. There was a significant difference in the hatching success of clutches in relation to order for *C.mydas* (H=9.69, p=0.047, n=96). Clutch 1 had a significantly lower hatching success than clutches 2 and 3. This relationship is illustrated in figure 4.3.4. However no such relationship was recorded with respect to hatchling emergence success and clutch order (H=5.21, p=0.268, n=95)

There were no significant differences in any of the parameters measured with respect to clutch number for *C.caretta;* inter-nesting period, (F=2.06, p=0.147, n=31), incubation period (F=2.44, p=0.096, n=59), clutch size (F=0.69, p=0.562, n=52), hatching success (F=0.67, p=0.518, n=51), hatchling emergence success (F=0.38, p=0.687, n=51), depth, either the top (F=0.00, p=0.997, n=52) or bottom(F=0.28, p=0.758, n=50), or hatchling length (F=0.17, p=0.679, n=33) or width (F=0.17, p=0.689, n=33).

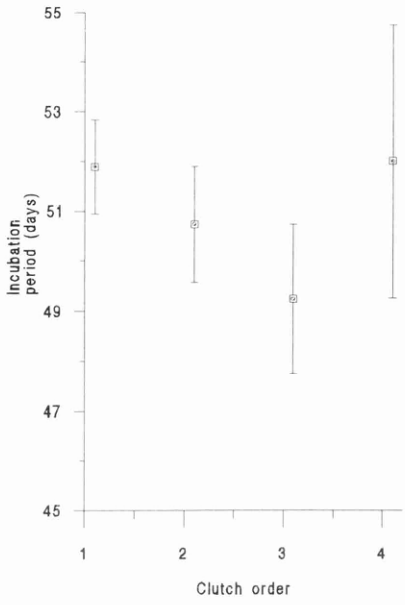


Figure 4.3.2. Mean incubation period of nests laid (days) with respect to their order of lay for *C.mydas* with 95% confidence intervals.

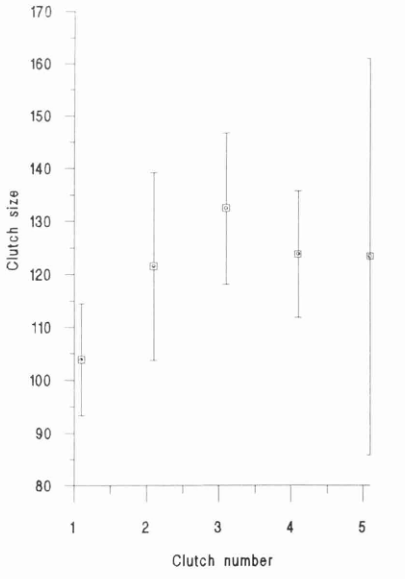


Figure 4.3.3. Mean clutch size of nests laid with respect to their order of lay for *C.mydas* with 95% confidence intervals.

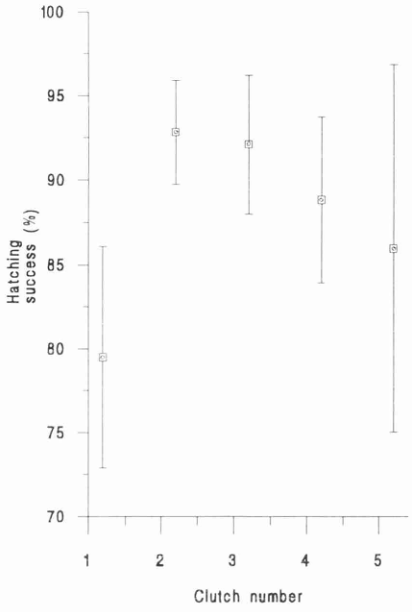


Figure 4.3.4. Mean hatching success of *C.mydas* nests in relation to the order of lay, with 95% confidence intervals.

4.4. Discussion

The recorded inter-nesting intervals for *C.mydas* are shorter that those of *C.caretta,* and although comparative data are unavailable for the Mediterranean population of *C.mydas*, at approximately 13 days, the interval accords with the world wide values reported by Miller (1996). Likewise, the slightly longer inter-nesting intervals for *C.caretta* in Cyprus fall within the global range, but interestingly are shorter than those recorded in Greece (Dodd 1988; Margaritoulis 1989). It is also noteworthy that although *C.mydas* females produce larger clutches, they have the shorter inter-nesting interval.

Many external and internal factors interact to influence ovarian ripening and clutch formation and the observed variations in the time between oviposition cannot be put down to one factor alone. Nevertheless, a shorter interval is recorded between nests of both species in Cyprus in comparison to females nesting at other sites in the Mediterranean, and one could speculate that the common factor is the prevailing climatic condition.

In 1994 the female population of *C.mydas* were larger in both length and width than in 1993 and 1995. In contrast *C.caretta* females were significantly larger in 1993 than in 1994. Several hypotheses can be put forward to account for these observations. It is feasible that environmental conditions, such as food availability and temperature, favoured larger females in these years or conversely, were unfavourable to smaller turtles. Another possibility is that different feeding assemblages exist in which females are different in size. These females may all be following the same nesting strategy so that they return in the same years to nest. Preliminary results of the tagging programme however, suggest that this is unlikely, for example some *C.mydas* females tagged in 1992 returned to nest in 1994 and others in 1995.

The mean overall curved carapace length of *C.mydas* females nesting in Northern Cyprus was 92.02 cm ($\pm$ 0.79, n=57). Comparing this value with data from Turkey collected by Baran and Kasparek (1989a), by way of a t-test, showed that *C.mydas* nesting in Turkey (90.1 cm) were significantly smaller than those nesting in Northern Cyprus ($t=2.163$, $p< 0.05$, n=98). However, curved carapace lengths of *C.mydas* females recorded nesting at Akyatan, Turkey, by Gerosa *et al* (1995) (92.1 cm), were not significantly different to those in Cyprus ($t=0.0687$, $p>0.05$, n=70).

The pooled mean carapace length for *C.caretta* females in Northern Cyprus (73.5 cm ± 0.56, n=72), was similar to that recorded in Turkey (73.1 cm) by Erk'akan (1993) (t=0.629, n= 121, p>0.05) but significantly smaller (t=15.36, n=144, p=0.01) than that recorded in Greece (83.1 cm) by Margaritoulis (1989). Other studies of *C.caretta* nesting in Turkey record similar curved carapace lengths of nesting females, indicating that there is a true difference in the size of females nesting in different parts of the eastern Mediterranean.

The shorter incubation periods recorded in Cyprus in the nests of *C.caretta* in comparison to those of *C.mydas* may be accounted for by variations in temperature, moisture content and the smaller size of the resultant offspring of *C.caretta.* In addition, pooled incubation periods recorded in Cyprus for both species were significantly shorter than those found in other studies of Mediterranean marine turtles. *C.mydas* nests laid in Cyprus (51.1 days ± 0.32, n=121) had a significantly shorter incubation period than that (53.8 days) recorded at Akyatan (t=5.45, n= 144, p=0.01) (Gerosa *et al* 1995). Similarly, the overall mean (48 days ± 0.27, n=115) incubation period of *C.caretta* nests in Cyprus was significantly shorter than that recorded in Greece (55.5 days: t=16.07, n=165, p=0.01; Margaritoulis 1989) and Turkey (59.3 days; t=30.42, n=162, p=0.01; Erk'akan 1993).

These shorter incubation periods of *C.mydas* and *C.caretta* nests are probably due to the prevailing warmer climate found in Cyprus. Other workers have shown that an increase in temperature decreases the incubation period of marine turtle nests (Billett *et al.* 1992; Mrosovsky *et al.* 1995). Incubation periods are considered further in chapter 6 and sand quality in chapter 8.

The overall mean clutch size of *C.mydas* in Cyprus (113.1 eggs ±2.84, n=130) was significantly larger than the 101.1 eggs on the Göksu Delta, Turkey, recorded by Peters and Verhoeven (1992). Gerosa *et al* (1995) recorded a mean clutch size of 122.9 eggs for *C.mydas* at Akyatan, Turkey. However, in that study, no variance was indicated and so statistical comparison was not possible. In contrast, the pooled mean clutch size of *C.caretta* nests in Cyprus (69.1 eggs ± 1.89, n=111) was significantly smaller than that recorded in Greece (t=20.3651, n=163, p=0.01; Margaritoulis 1989) and Turkey (t=2.066, n=346, p=0.05; Erk'akan 1993) indicating that those found in Cyprus may indeed by smaller than those elsewhere in the Mediterranean. Certainly there is again a clear difference between Cyprus and

Greece. However, clutch sizes laid at Alagadi in 1993 were significantly larger than those laid in 1994 and 1995 this larger mean (79.3) being greater than that recorded by Erk'akan (1993) in Turkey.

Many reasons exist for the low overall success of nests seen in some years (table 4.3.3.), including predation, human disturbance, infestation and inundation. Due to their shallower nature, *C.caretta* nests are often more vulnerable to such threats and this may, in itself, explain why in the three years of this study, more *C.mydas* nests hatched and had a higher hatching success than those of *C.caretta*. The 1995 season was particularly bad for *C.caretta* nests, mainly due to the high levels of predation that occurred that year. The fate of nests will be discussed further in chapter 8.

The overall mean hatching success of *C.mydas* clutches of 85.8 % (± 1.41, n=130) is significantly higher than that recorded by Peters and Verhoeven (1992) on the Göksu Delta in Turkey ($t=4.614$, $n=139$, $p=0.01$). There was however no significant difference between the mean hatching success of *C.caretta* clutches in Cyprus (77.6 % ± 2.04, n=109) and Turkey ($t=0.207$, $n= 142$, $p>0.05$; Peters & Verhoeven 1992). Margaritoulis *et al.* (1995) quote a mean hatching success for *C.caretta* in Greece of 73.4%, however no variance was given to enable a statistical comparison. The hatchling emergence success is only, on average, slightly less than the hatching success implying that hatchlings at Alagadi have few problems emerging from the sand.

The literature provides no data for *C.mydas* hatchling sizes in the Mediterranean. However in this investigation, a significant difference in the lengths of *C.mydas* hatchlings was recorded, with those in 1994 significantly longer than in 1993 and 1995. As with clutch sizes and depths of nests it may be that this observation reflects adult sizes. *C.caretta* hatchling lengths in Cyprus (overall mean 4.07, ± 1.19 n=508) were not significantly different to those recorded by Sutherland (1985) in Greece ($t=0.571$, $n=729$, $p>0.05$). However, annual variation, as recorded in *C.mydas*, was not recorded in *C.caretta* hatchlings suggesting that in this species a relationship between adult and hatchling size may not exist.

The nesting and hatching parameters recorded in *C.caretta* nests were not found to vary significantly with respect to the number of clutches a female had previously laid; this may be related to the fact that few females lay more than 3 clutches. In contrast, *C.mydas*

incubation periods, clutch sizes and hatching success were all found to vary significantly with clutch order. The mean incubation periods of *C.mydas* nests, were significantly shorter in the third clutch than the first or fifth which is probably related to the fact that the former nests, being laid in high summer, will have been subject to higher temperatures throughout incubation.

The mean size of the first clutch laid by *C.mydas* females was significantly smaller than the third and, a decline, although not significant, is seen in the mean clutch sizes thereafter. Carr (1975) reported similar results with *C.mydas* laying fewer eggs in the first and last clutch of a season. However, Bjorndal & Carr (1989), reported an increase in clutch sizes as the season progressed at a population level but not at an individual level. This variation in clutch size is possibly a reflection of diminishing resources of females.

It is to be expected that, analogous to the domestic fowl (Solomon 1991) the ovary becomes less efficient toward the end of the laying year entering a quiescent phase. In populations of domestic breeding fowl, hatching success also diminishes with increasing bird age and is also less predictable during the early phase of egg production. Likewise, in marine turtle populations clutches laid at either end of the season are less successful, possibly for the same reasons. In addition unsettled weather conditions at each end of the season may also influence the process.

The results presented in this chapter, although the first of their kind in the Mediterranean, are not definitive. However, they do provide baseline information on the nesting ecology of these two species nesting side by side on the same stretch of beach, which increases their value. In chapter 5 the relationships that exist between the measurements presented in this section are examined which may help to explain some of the annual variation recorded.

Chapter 5 - The Influence of Female Size on the Resultant Reproductive Output.

5.1. Introduction

Many optimality models suggest a relationship between female resources and offspring fitness. Some species are thought to follow the strategy of investing in many small offspring whereas others have fewer, larger offspring (McGinley 1989). There may be a trade off, in marine turtles, between the number of clutches laid, clutch size and hatchling size. By laying a larger number of clutches a female may decrease the chance of all of her eggs being discovered by predators, however each emergence uses up energy reserves and may incur a risk of predation. Alternatively, offspring from larger clutches may experience a higher probability of survival due to predator satiation and larger hatchlings may have a greater chance of escaping predation. In the case of marine turtles then, larger clutches may increase initial survival, and once the hatchlings are in the water, larger individuals may also have a higher chance of survival.

McGinley (1989) suggests that offspring size will only vary within a species if there is a relationship between size and fitness. He showed that social facilitation increased hatchling speed which acted to reduce predation levels. McGinley (1989) does, however, state that the benefits of producing a large clutch also depend on the synchrony of hatching, suggesting that species which nest in groups or 'arribadas' would not show this correlation between female and hatchling size.

If larger females were able to allocate more resources to their offspring it would be expected that they would do so. However, anatomical and physiological factors may constrain this. For example, egg size may be limited by the size of the cloaca or oviduct. The size of the pelvic girdle has been shown to limit the maximum egg size produced in some species of freshwater turtle (Congdon *et al.* 1983). Alternatively, other factors such as moisture and temperature conditions in the nest may also affect hatchling size. Janzen (1993) incubated 17 clutches of *Chelydra serpentine,* the common snapping turtle, in 'wet' substrates, producing large hatchlings during longer incubation periods than 'dry' substrates, which produced smaller hatchlings over shorter incubation periods. He found that hatchling size was unrelated to hatching success of a nest. He then undertook a mark and recapture study using drift nets and this showed survivorship to be significantly higher in larger hatchlings.

Some studies suggest that sea turtles do not conform to the direct correlation between clutch size and body size, although clutch and egg size appear to be correlated (Ehrhart 1982). Others support the relationship between female size and clutch size (Chen & Cheng 1995; Hays & Speakman 1991; Loop *et al.* 1995). Obviously this relationship may not exist in all species of marine turtle. It might be expected that larger species of marine turtles would have a larger clutch size. This is true for most species but not for *D.coriacea* or *N.depressa* (Ehrhart 1982). These have much smaller clutches than would be expected for their relative sizes. The larger size of the eggs of *Dermochelys,* in comparison to other species, may explain some of this variation and it has been suggested that the small clutch size of *N.depressa* may be a limitation of the capacity to hold many eggs due to a flattened body shape (Ehrhart 1982).

When examining female reproductive output it is also important to take into account the number of clutches a female lays in a season. Limpus (1996a) found that in Australia, the number of clutches laid in a season by *C.caretta* was poorly correlated to female size and her past breeding history. Visual examinations of ovaries during and after the breeding season demonstrated that the length of the breeding migration had profound effects on the seasonal egg production of individual turtles. Females migrating from feeding areas close to nesting beaches successfully ovulated nearly all mature follicles present in the ovary, whereas those migrating from a distant feeding ground underwent atresia of one or more clutches of mature follicles.

Reproductive output can be gauged as a function of the number and size of clutches laid by a female per nesting season, the size of the eggs in these clutches, the size and number of hatchlings produced, the interval between nesting seasons and the length of reproductive life. With so many variables, all having an effect on the reproductive output of females, it is likely that there will be a complex relationship limiting or controlling such factors. Many different strategies may be in operation.

This chapter examines the effect of female size on the number and size of clutches laid, the size of hatchlings produced, and the success of nests. In addition, other factors may also affect reproductive output. The depth to which females can dig their nest may be a constraint of female size and affect the incubation period of the nest, the success of the nest and, due to the physical environment of the nest, possibly hatchling size. These factors are therefore also examined in this chapter.

5.2. Methodology

The methodology used in collecting the data examined in this chapter is given in chapter 2. Prior to analysis, a substantial amount of data manipulation was necessary and is described in this section.

Where a female nested more than once in a season, and variation existed in the curved carapace measurements recorded, mean length and width were calculated for use in analyses. One female nested in more than one year of this study and was removed from the data set. This, as previously mentioned, was to avoid the problem of pseudoreplication (Hurlbert 1984).

In an effort to gain one measure to represent female size, Principal Components Analysis (PCA) was used. This is a data reduction technique which takes account of the relationship between the curved carapace length and width and calculates coefficients which account for a proportion of the variance. Thus, in analysis, this value can be used as a measure of female size. The measure obtained by PCA is referred to as $PCA_{adult\ size}$ throughout the remainder of this chapter. Similarly the term $PCA_{hatchling\ size}$ is also used to refer to the result obtained using PCA to compare hatchling straight carapace length and width. A comparison of the relationship between adult and hatchling carapace length and width of the two species is made using Analysis of Covariance.

For each species, two data sets were analysed. One included a comparison of female size with the day of the season on which she had laid her first clutch, the number of clutches she laid in a season, her mean clutch size and mean inter-nesting interval. The day of the season is taken as day 1 being the date on which the first nest, for each species, was recorded in that year. For the analysis of the number of clutches a female laid, the corrected values, calculated in chapter 3, were used rather than the observed number of clutches recorded.

The second data set examined includes female size and the results recorded from one nest for each female; clutch size, hatching success, hatchling emergence success, hatchling size, depth of the nest and the incubation period of a nest. This nest was selected at

random. This eliminates the error of pseudoreplication (Hurlbert 1984). This does however mean that data sets are reduced.

In an effort to increase normality, hatching success and hatchling emergence success were *arc sine* transformed for use in analyses.

In most analyses, linear regression was used to compare the relationship between two variables. In some instances, when comparing the factors affecting the number of clutches a female laid, a one way ANOVA was performed for normally distributed data, or a Kruskal-Wallis if the data were non-parametric. This is specified in the relevant section of the results. After a parametric test had been conducted the residuals were tested to ensure they showed normality before the results were deemed acceptable.

5.3. Results

5.3.1. The effect of female size on reproduction.

Statistical results discussed in this section are given in table 5.3.1. for *C.mydas*, table 5.3.2. for *C.caretta,* or in the text in the relevant sections. Principal Components Analysis was found to account for 90.5% of the variance of *C.mydas* data and 93% of *C.caretta* data through the scores calculated for axis 1.

5.3.1.1. Chelonia mydas

The relationship between the three indices of female size ($PCA_{adult\ size}$, curved carapace length and curved carapace width) and the day of the season on which females laid their first clutch were examined using regression analysis. No significant relationships were recorded in the case of any of the three indices of female size. A one way ANOVA was used to examine whether the number of clutches a female laid in any one season varied in relation to female size. None of the measurements were related to the number of clutches a female laid. Neither did any of the three indices of female size have any significant relationship with the mean inter-nesting interval (regression analysis).

In chapter 4 a significant difference was recorded between clutch sizes in the three years of this study and was attributed to the observed variation in female size. However, here mean clutch size is used in analyses and thus there is only one entry for each female. Before advancing further mean clutch size was examined for yearly variations using a one way ANOVA and did not vary significantly ($F=3.19$, $p=0.052$, $n=48$). This data set could therefore be compared to female size. Using regression analysis, it was found that all three indices of female size were significantly related to mean clutch size. All these relationships were positive. Female $PCA_{adult\ size}$ was the most significant of the three and is illustrated in figure 5.3.1.

A significant relationship was not recorded between any of the three indices of size of nesting females and $PCA_{hatchling\ size}$ or straight carapace width of hatchlings (regression analysis). However, significant relationships were recorded between hatchling straight carapace length and all three measures of female $PCA_{adult\ size}$, length and width. The positive relationship between female $PCA_{adult\ size}$ and hatchling straight carapace length is illustrated in figure 5.3.2., it being the strongest of the relationships recorded.

Parameter	*Female $PCA_{adult\ size}$*			*Curved carapace length (cm)*			*Curved carapace width (cm)*		
	Statistic	*Probability*	*N*	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Day of the season of first nest	F=2.82	p=0.063.	n=54	F=4.0	p=0.051	n=54	F=3.75	p=0.058	n=54
Number of clutches	F=1.44	p=0.235	n=54	F=1.12	p=0.358	n=54	F=1.61	p=0.187	n=54
Mean Inter-nesting period (days)	F=0.18	p=0.672	n=35	F=0.39	p=0.535	n=35	F=0.03	p=0.859	n=35
Mean clutch size	F=33.01	p< 0.0005*	n=48	F=32.14	p< 0.0005*	n=48	F=26.11	p< 0.0005*	n=48
Hatchling;									
$PCA_{hatchling\ size}$	F=2.78	p=0.128	n=28	F=3.18	p=0.086	n=28	F=1.53	p=0.228	n=28
Straight carapace length(cm)	F=6.58	p=0.016*	n=28	F=6.53	p=0.017*	n=28	F=5.16	p=0.032*	n=28
Straight carapace width(cm)	F=0.28	p=0.599	n=28	F=0.71	p=0.408	n=28	F=0.05	p=0.829	n=28
Hatching success (transformed)	F=0.01	p=0.942	n=48	F=0.01	p=0.920	n=48	F=0.01	p=0.919	n=48
Hatchling emergence success (transformed)	F=0.25	p=0.621	n=48	F=0.13	p=0.718	n=48	F=0.32	p=0.575	n=48
Incubation period (days)	F=0.17	p=0.679	n=49	F=0.03	p=0.865	n=49	F=0.81	p=0.374	n=49
Depth of the egg chamber;									
top	F=3.63	p=0.063	n=48	F=4.81	p=0.033*	n=48	F=2.1	p=0.154	n=48
bottom	F=6.69	p=0.013*	n=48	F=8.14	p=0.007*	n=48	F=4.31	p=0.044*	n=48

Table 5.3.1. The relationship between the carapace dimensions of *C.mydas* nesting females and parameters related to reproductive output.

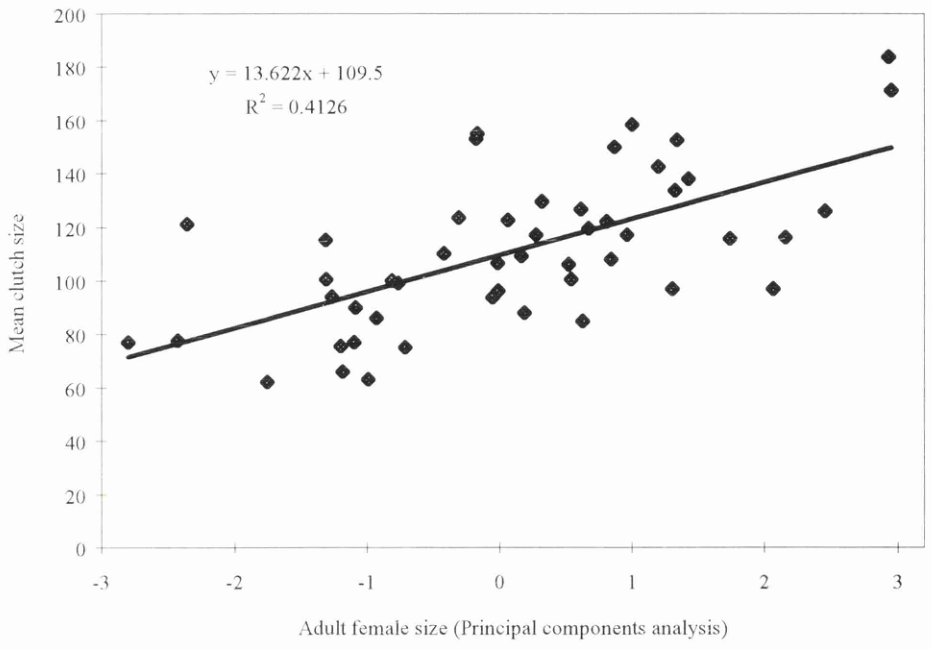


Figure 5.3.1. The relationship between *C.mydas* $PCA_{adult\ size}$ and mean clutch size.

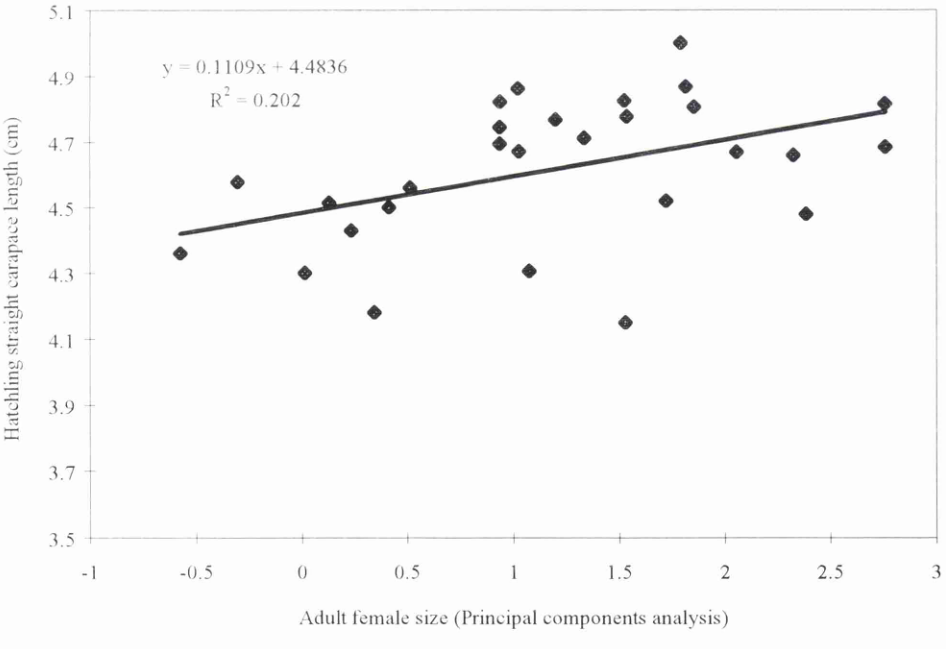


Figure 5.3.2. The relationship between *C.mydas* $PCA_{adult\ size}$ and the straight carapace length of hatchlings (cm) produced.

Neither of the three measures of female $PCA_{adult\ size}$, length or width were significantly related to either the hatching success or hatchling emergence success of a clutch.

In chapter 4, the depth of nests was significantly different between the three years. However, when testing this smaller data set using a one way ANOVA no such difference was recorded between either the top of the egg chamber ($F=2.82$, $p=0.07$, $n=48$), or the bottom ($F=2.17$, $p=0.127$, $n=45$). The relationship between female size and the depth of a nest could therefore be examined using regression analysis. A significant relationship was recorded between the depth to the top of the egg chamber and female curved carapace length but not with female $PCA_{adult\ size}$ or width. All three indices of female size, were however significantly related to the depth of the bottom of the egg chamber. Figure 5.3.3. illustrates the relationship between female curved carapace length and the depth of both the top and bottom of the egg chamber.

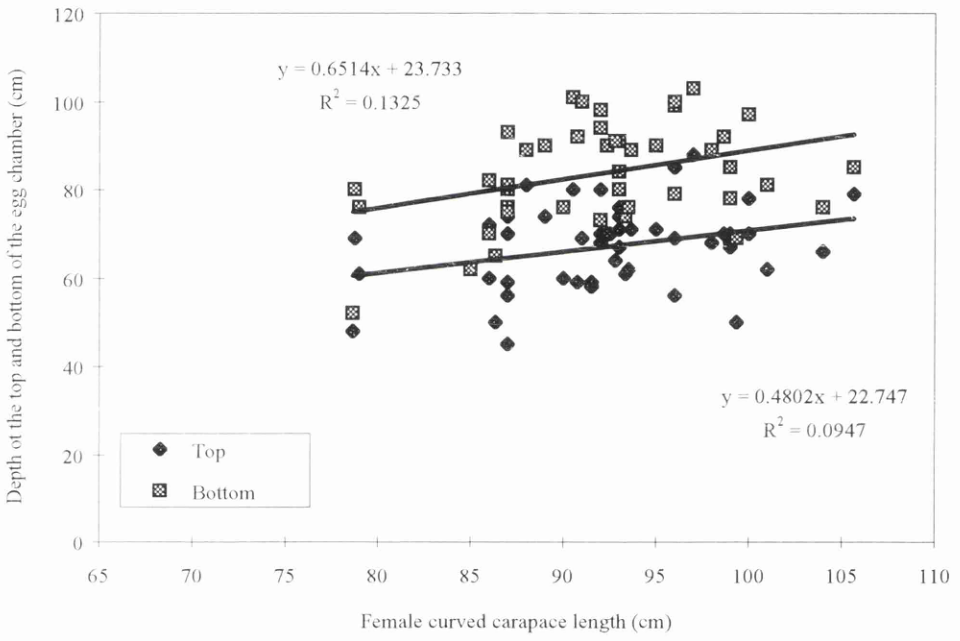


Figure 5.3.3. The relationship between the length of the carapace of nesting *C.mydas* females and the depth to the top and bottom of the egg chamber of her nest.

Using regression analysis, no significant relationships were recorded between female size and the length of the incubation period of a clutch. Neither were any significant relationships recorded between the length of the incubation period and the depth of the top ($F=3.44$, $p=0.07$, $n=47$) or bottom of the egg chamber of a nest ($F=0.00$, $p=0.989$, $n=47$). In addition, no significant relationship was recorded between the size of the clutch laid and the resultant incubation period ($F=0.02$, $p=0.887$, $n=48$).

5.3.1.2. C.caretta

Regression analysis showed that the day of the season on which a female laid her first clutch was not significantly related to $PCA_{adult\ size}$, or width. Only the curved carapace length showed a significant relationship with the day of the season. This was negative with larger females being the first nesters in a season (figure 5.3.4.).

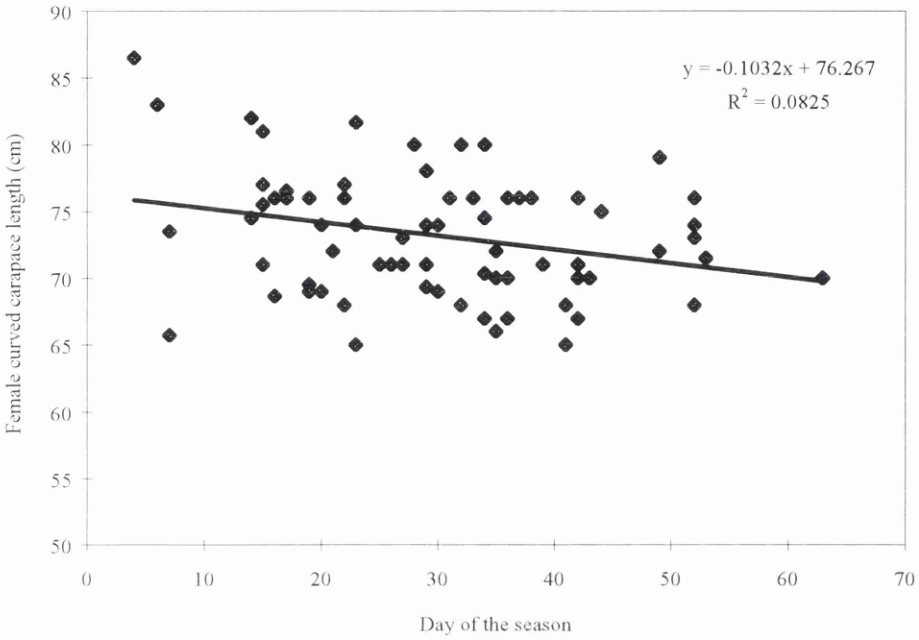


Figure 5.3.4. The relationship between the curved carapace length of nesting *C.caretta* females and the day of the season on which they laid their first clutch.

Regression analysis showed that none of the three indices of female size were shown to have any effect on; the mean inter-nesting interval, hatchling size, hatching success or hatchling emergence success and either the depth to the top or bottom of the egg chamber. A one way ANOVA was used to determine whether females that laid more clutches were larger as, after conducting regression analyses on these data, the residuals did not show normality. No such relationship was recorded.

Although clutch sizes were found to be significantly different with respect to the three years of this study, mean clutch sizes of individual females used in these analyses did not vary between the years ($F=1.87$, $p=0.167$, $n=45$). Highly significant positive relationships were recorded between all indices of size and the mean size of the clutches laid (regression). Of the three measures of size, the curved carapace width of females showed the strongest relationship and is illustrated in figure 5.3.5.

Parameter	*Female $PCA_{adult\ size}$*			*Curved carapace length (cm)*			*Curved carapace width (cm)*		
	Statistic	*Probability*	*N*	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Day of the season of first nest	F=3.54	p=0.064	n=71	F=6.2	p=0.015*	n=71	F=1.36	p=0.248	n=71
Number of clutches	F=0.69	p=0.564	n=71	F=0.73	p=0.537	n=71	F=0.6	p=0.564	n=71
Mean Inter-nesting period (days)	F=2.4	p=0.133	n=29	F=1.25	p=0.274	n=29	F=3.63	p=0.067	n=29
Mean clutch size	F=14.53	p<0.0005*	n=41	F=11.3	p=0.002*	n=41	F=15.07	p< 0.0005*	n=41
Hatchling;									
$PCA_{hatchling\ size}$	F=0.57	p=0.455	n=29	F=0.22	p=0.639	n=29	F=0.89	p=0.354	n=29
Straight carapace length(cm)	F=0.75	p=0.395	n=29	F=0.47	p=0.498	n=29	F=0.91	p=0.349	n=29
Straight carapace width(cm)	F=0.35	p=0.556	n=29	F=0.05	p=0.824	n=29	F=0.75	p=0.395	n=29
Hatching success (transformed)	F=0.28	p=0.602	n=40	F=0.7	p=0.408	n=41	F=0.03	p=0.860	n=41
Hatchling emergence success (transformed)	F=0.19	p=0.663	n=40	F=0.32	p=0.574	n=40	F=0.08	p=0.782	n=40
Incubation period (days)	F=4.28	p=0.045*	n=41	F=6.23	p=0.017*	n=41	F=2.27	p=0.140	n=41
Depth of the egg chamber;									
top	F=0.01	p=0.943	n=40	F=0.01	p=0.906	n=40	F=0.00	p=0.985	n=40
bottom	F=0.13	p=0.716	n=40	F=0.02	p=0.889	n=40	F=0.33	p=0.571	n=40

Table 5.3.2. The relationship between the carapace dimensions of *C.caretta* nesting females and parameters related to reproductive output.

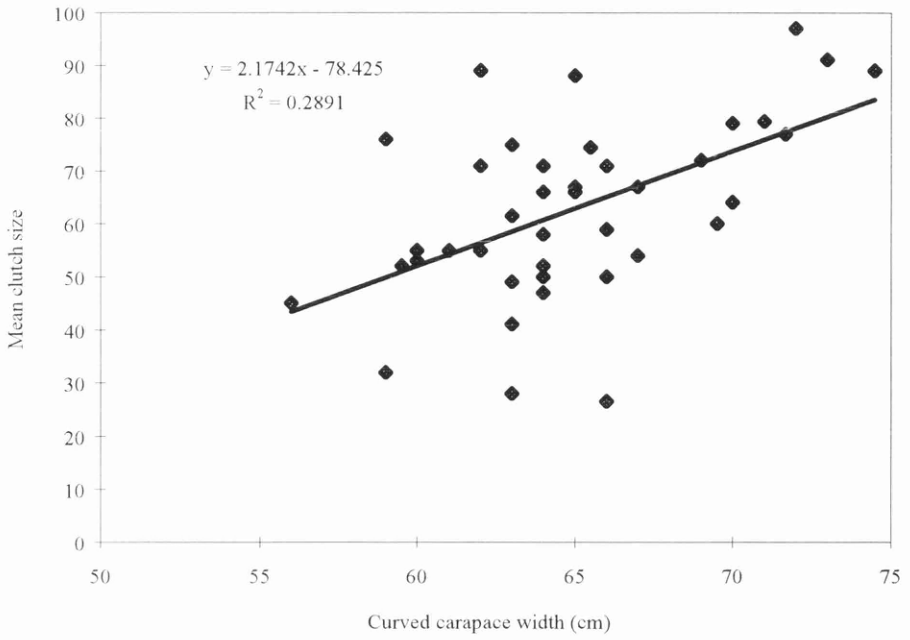


Figure 5.3.5. The relationship between the curved carapace width of nesting *C.caretta* females and the mean size of clutches laid.

With respect to the incubation period of a nest, both female $PCA_{adult\ size}$ and length were found to be significantly related to the incubation period of a nest although curved carapace width was not. The significant relationships recorded in these instances were positive, with nests of larger females having longer incubation periods. The relationship between female curved carapace length and the incubation period of nests is illustrated in figure 5.3.6. No relationship was recorded between the incubation period of a nest and either the depth to the top ($F=0.00$, $p=0.990$, $n=41$) or bottom of the egg chamber ($F=0.25$, $p=0.621$, $n=41$). Clutch size also had no significant relationship with the incubation period of a nest ($F=0.31$, $p=0.580$, $n=41$).

Figure 5.3.7. illustrates the relationship between curved carapace length and width of both species to aid interpretation of the results recorded with respect to the three indices of female size. Analysis of Covariance was used to compare these two regression lines. No significant differences were found between either the slope ($F=1.15$, $p=0.285$, $n=125$) or the elevation ($F=2.08$, $p=0.152$, $n=125$) of the two lines.

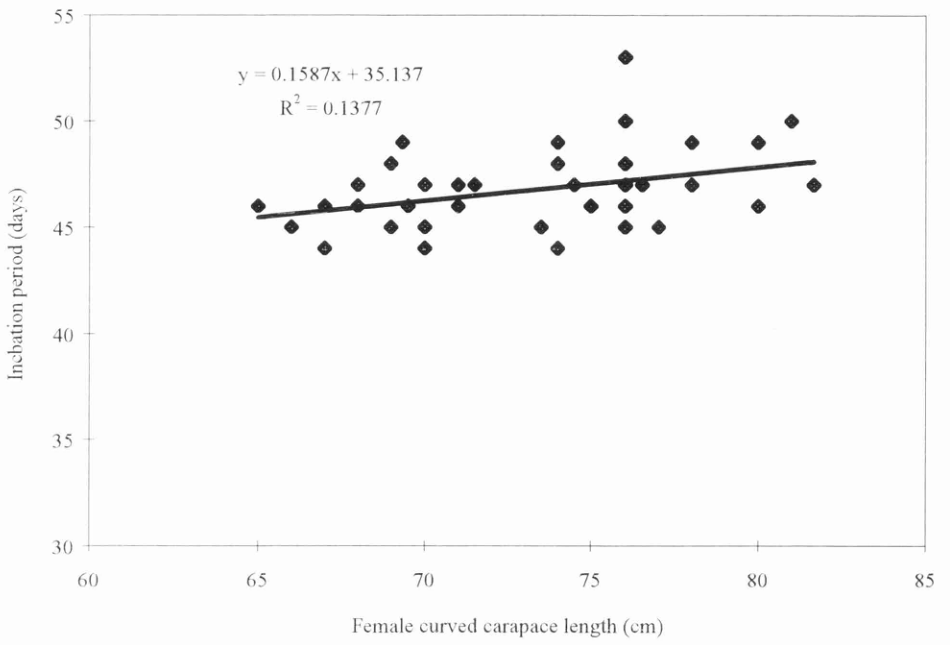


Figure 5.3.6. *C.caretta* female carapace length and the incubation period of nests.

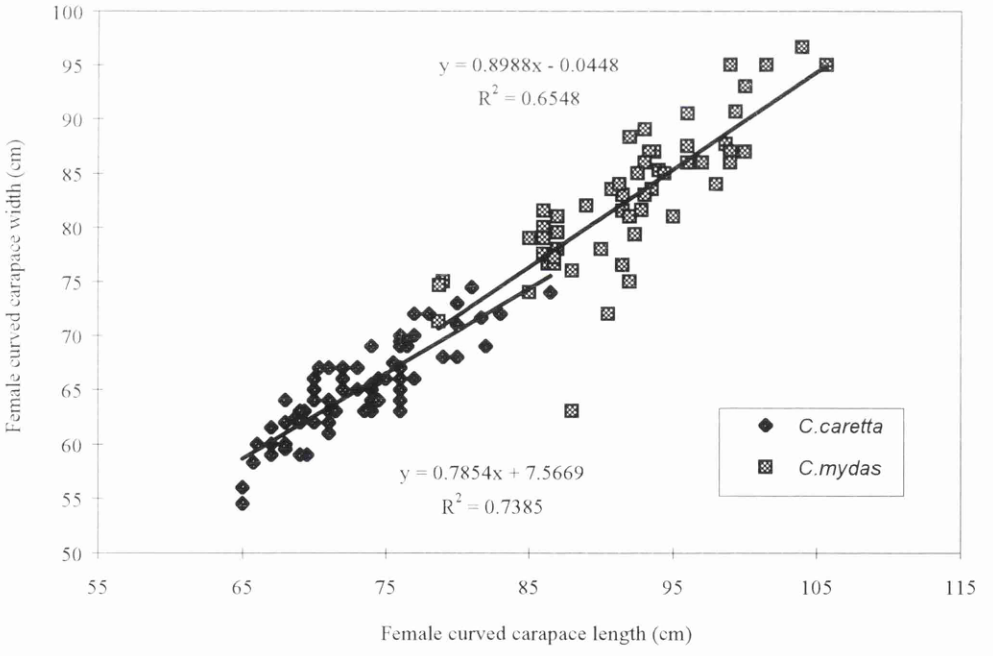


Figure 5.3.7. The relationship between curved carapace length and width of *C.mydas* and *C.caretta* nesting females.

5.3.2. The effect of season, inter-nesting interval and clutch size on the number of clutches a female laid.

Results discussed in this section are given in table 5.3.3. for *C.mydas* and table 5.3.4. for *C.caretta* or in brackets in the relevant sections.

5.3.2.1. C.mydas

Due to the non-parametric nature of the data, a Kruskal-Wallis test was used to examine whether there was a significant difference between the number of clutches laid by females in each of the three years of this study. A significant difference was recorded with females in 1995 laying significantly more clutches than in 1993 or 1994. A significant relationships was also recorded between the number of clutches a female laid and the day of season on which she laid her first nest. Females nesting early in the season laid more clutches than those that started nesting later.

	Number of clutches laid		
Parameter	*Statistic*	*Probability*	*N*
Year	H=7.6	p=0.023*	n=54
Day of the season of first nest	F=6.55	p=0.013*	n=54
Mean inter-nesting period	F=17.71	p< 0.0005*	n=33
Mean clutch size	F=1.06	p=0.387	n=47

Table 5.3.3. Results of analyses to compare the number of clutches a *C.mydas* females laid in a season and the year, day of the season of first nest, mean inter-nesting interval and mean clutch size.

The mean inter-nesting period of a female, using regression analysis, was found to be significantly related to the number of clutches laid. The shorter the inter-nesting interval the greater number of clutches laid (figure 5.3.8.).

Mean clutch size (F=1.06, p=0.387, n=47) was not related to the number of clutches a female laid. Mean clutch size was also not significantly related to the mean inter-nesting interval of a female (F=0.00, p=0.987, n=32). Neither was the inter-nesting interval significantly related to either the size of the clutch laid previously (F=0.00, p=0.993, n=53) or subsequently (F=0.78, p=0.381, n=52).

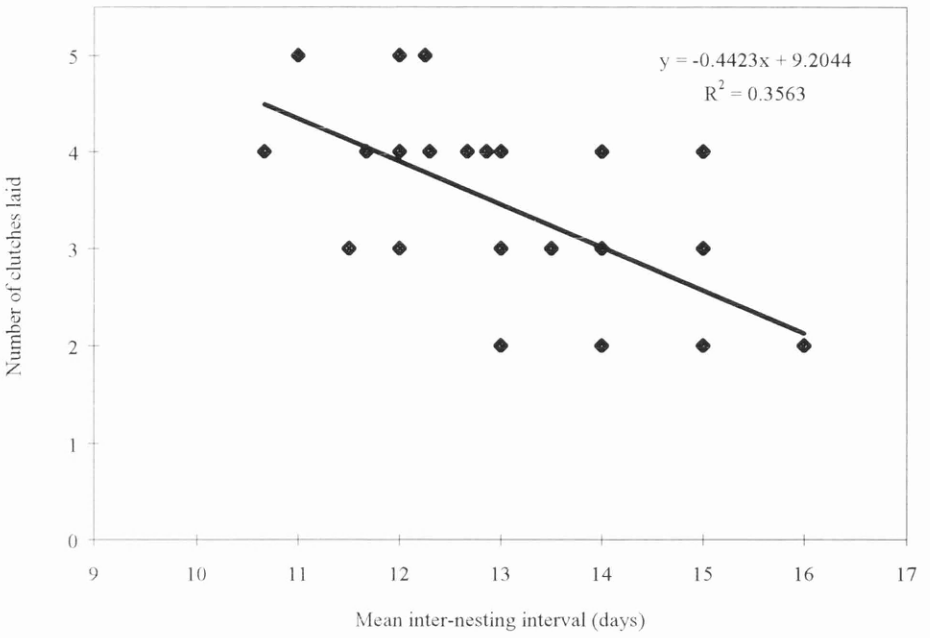


Figure 5.3.8. The relationship between the mean inter-nesting period of individual *C.mydas* females and the number of clutches laid in a season.

5.3.2.2. C.caretta

Due to the non-parametric nature of the data a Kruskal-Wallis test was used to examine whether there was a significant difference between the number of clutches laid by females in each of the three years of this study. No significant differences were recorded between the medians in the three years. A significant relationship was however recorded, using a one way ANOVA, between the number of clutches laid and the day of the season on which the first nest was laid. Again more clutches were laid by females laying their first clutch earlier in the season.

Similarly a one way ANOVA was used to examine whether there was a significant relationship between the number of clutches a female laid and either her mean inter-nesting period or mean clutch size. No such significant relationships were recorded. Neither was a significant relationship recorded between the mean inter-nesting interval and mean clutch size ($F=1.41$, $p=0.249$, $n=21$) or the inter-nesting interval and the size of the clutch laid previously ($F=0.52$, $p=0.485$, $n=13$). Not enough data were available to compare the inter-nesting period and the size of clutches laid subsequently.

	Number of clutches laid		
Parameter	*Statistic*	*Probability*	*N*
Year	H=0.18	p=0.912	n=80
Day of the season of first nest	F=6.4	p=0.001*	n=80
Mean inter-nesting period	F=0.45	p=0.643	n=28
Mean clutch size	F=0.65	p=0.585	n=45

Table 5.3.4. Results of analyses to compare the number of clutches a *C.caretta* females laid in a season and the year, day of the season of first nest, mean inter-nesting interval and mean clutch size.

5.3.3. The effect of clutch size, incubation period, hatchling size and the depth of the nest on the resultant success of a nest.

Results discussed in this section are given in table 5.3.5. for *C.mydas* and table 5.3.6. for *C.caretta* or in brackets in the relevant sections.

5.3.3.1. C.mydas

No significant relationships were recorded between either the clutch size, incubation period, any of the three indices of hatchling size or depth of the egg chamber and either the hatching success or hatchling emergence success of a nest.

	Hatchling success			*Hatchling emergence success*		
Parameter	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Clutch size	F=0.06	p=0.802	n=48	F=1.17	p=0.285	n=48
Incubation period (days)	F=2.25	p=0.140	n=48	F=0.85	p=0.366	n=48
Hatchling;						
$PCA_{hatchling\ size}$	F=0.03	p=0.868	n=28	F=0.76	p=0.392	n=28
Straight carapace length(cm)	F=0.02	p=0.893	n=28	F=0.2	p=0.661	n=28
Straight carapace width(cm)	F=0.00	p=0.996	n=28	F=1.1	p=0.305	n=28
Depth of the egg chamber;						
top	F=0.88	p=0.353	n=48	F=0.8	p=0.377	n=48
bottom	F=0.96	p=0.333	n=48	F=1.15	p=0.290	n=48

Table 5.3.5. The relationship between the success of *C.mydas* nests and clutch size, incubation period, hatchling size and depth of the nests.

5.3.3.2. C.caretta

No significant relationships were recorded between the clutch size and incubation periods of a nest and the hatching success or hatchling emergence success of a nest.

	Hatchling success			*Hatchling emergence success*		
Parameter	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Clutch size	F=0.02	p=0.894	n=41	F=0.08	p=0.779	n=41
Incubation period (days)	F=0.73	p=0.398	n=40	F=0.57	p=0.455	n=40
Hatchling;						
$PCA_{hatchling\ size}$	F=18.3	p<0.0005*	n=29	F=10.88	p=0.003*	n=29
Straight carapace length(cm)	F=20.33	p<0.0005*	n=29	F=10.55	p=0.003*	n=29
Straight carapace width(cm)	F=13.65	p=0.001*	n=28	F=8.88	p=0.006*	n=29
Depth of the egg chamber;						
top	F=11.48	p=0.002*	n=41	F=7.61	p=0.009*	n=41
bottom	F=4.28	p=0.046*	n=41	F=1.41	p=0.243	n=41

Table 5.3.6. The relationship between the success of *C.caretta* nests and clutch size, incubation period, hatchling size and depth of the nests.

However, both hatching success and hatchling emergence success were significantly related to all three indices of hatchling size. These relationships were all positive with larger hatchlings being found in nests with a higher success. In all cases a stronger relationship was recorded with hatching success than hatchling emergence success. The strongest relationships was recorded between hatching success and hatchling straight carapace and is illustrated in figure 5.3.9.

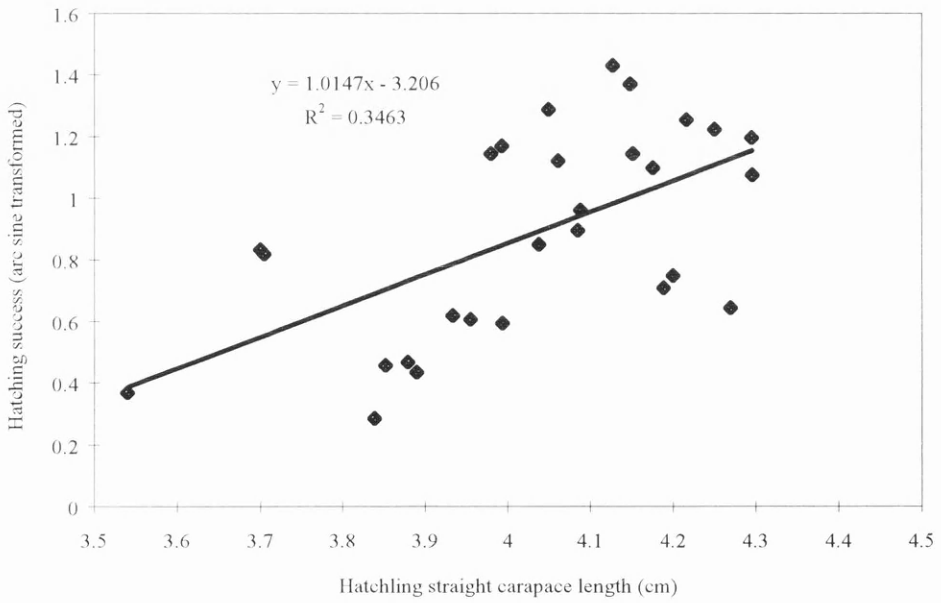


Figure 5.3.9. The straight carapace length of *C.caretta* hatchlings and the resultant hatching success of a nest.

A significant positive relationship was also recorded between the depth to the top and the bottom of the egg chamber and the hatching success of a nest. Similarly a positive relationship was recorded between the hatchling emergence success of a nest and the depth to the top of the egg chamber although not between the former and the depth to the bottom of the nest. The relationship between hatching success and the depth of the nest is illustrated in figure (figure 5.3.10.).

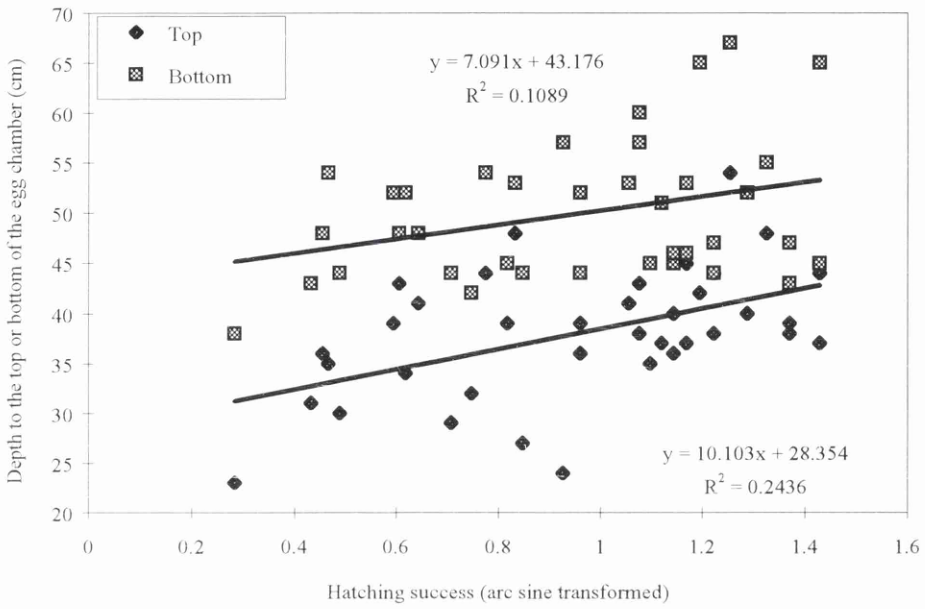


Figure 5.3.10. The depth to the top and bottom of the egg chamber of *C.caretta* nests and the resultant hatching success of the nest.

5.3.4. The effect of clutch size, incubation period and the depth of the nest on hatchling size.

Results discussed in this section are given in table 5.3.7. for *C.mydas* and table 5.3.8. for *C.caretta* or in brackets in the relevant sections.

5.3.4.1. C.mydas

A significant positive relationship was recorded between the size of a clutch and the $PCA_{hatchling\ size}$, straight carapace length and width of hatchlings. The strongest of these three relationships was that between clutch size and hatchling size and is illustrated in figure 5.3.11.

Parameter	*Hatchling PCA$_{hatchling\ size}$*			*Straight carapace length (cm)*			*Straight carapace width (cm)*		
	Statistic	*Probability*	*N*	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Clutch size	F=18.53	p<0.0005*	n=28	F=11.78	p=0.002*	n=28	F=12.81	p=0.001*	n=28
Incubation period (days)	F=0.03	p=0.869	n=28	F=0.11	p=0.737	n=28	F=0.31	p=0.582	n=28
Depth of the egg chamber;									
top	F=0.03	p=0.870	n=27	F=0.07	p=0.8	n=27	F=0.00	p=0.952	n=27
bottom	F=3.78	p=0.063	n=27	F=3.82	p=0.062	n=27	F=2.20	p=0.150	n=27

Table 5.3.7. The results of analyses to investigate the effect of other factors (clutch size, incubation period and depth of the nest) on the size of *C.mydas* hatchlings.

Parameter	*Hatchling PCA$_{hatchling\ size}$*			*Straight carapace length (cm)*			*Straight carapace width (cm)*		
	Statistic	*Probability*	*N*	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Clutch size	F=0.53	p=0.472	n=29	F=0.18	p=0.673	n=29	F=0.87	p=0.359	n=29
Incubation period (days)	F=0.93	p=0.343	n=29	F=0.39	p=0.535	n=29	F=1.4	p=0.247	n=29
Depth of the egg chamber;									
top	F=0.01	p=0.925	n=29	F=0.01	p=0.927	n=29	F=0.01	p=0.928	n=29
bottom	F=0.26	p=0.613	n=29	F=0.22	p=0.646	n=29	F=0.26	p=0.614	n=29

Table 5.3.8. The results of analyses to investigate the effect of other factors (clutch size, incubation period and depth of the nest) on the size of *C.caretta* hatchlings

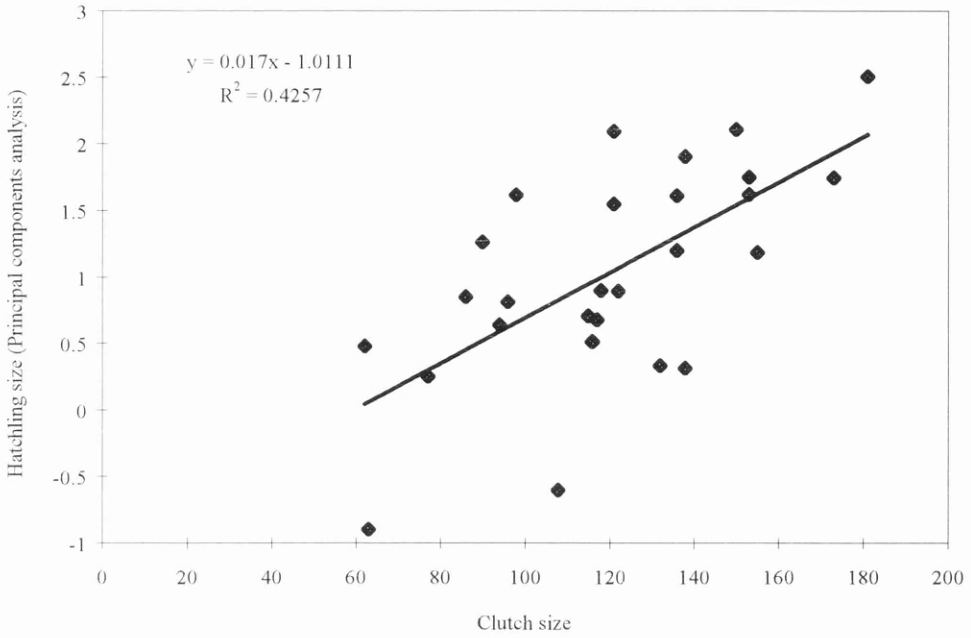


Figure 5.3.11. The relationship between the size of clutches laid by females and the size of *C.mydas* hatchlings produced.

There was no effect of the length of the incubation period on the size of hatchlings produced. There were also no significant relationships recorded between hatchling size and the depth, to the top or bottom, of the egg chamber.

5.3.4.2. C.caretta

Regression analysis was used to examine the relationship between hatchling size, ($PCA_{hatchling\ size}$, curved carapace length and width) and the other parameters given in table 5.3.8., where the results of these analyses can be found. No significant relationship was recorded between hatchling size and clutch size, incubation period or depth of the egg chamber, either to the top or bottom.

Figure 5.3.12. is included to illustrate the relationship between straight carapace length and width of *C.mydas* and *C.caretta* hatchlings. Analysis of Covariance was used to compare these two regression lines. No significant differences were found between either the slope ($F=1.98$, $p=0.165$, $n=55$) or the elevation ($F=0.1$, $p=0.749$, $n=55$) of the two lines.

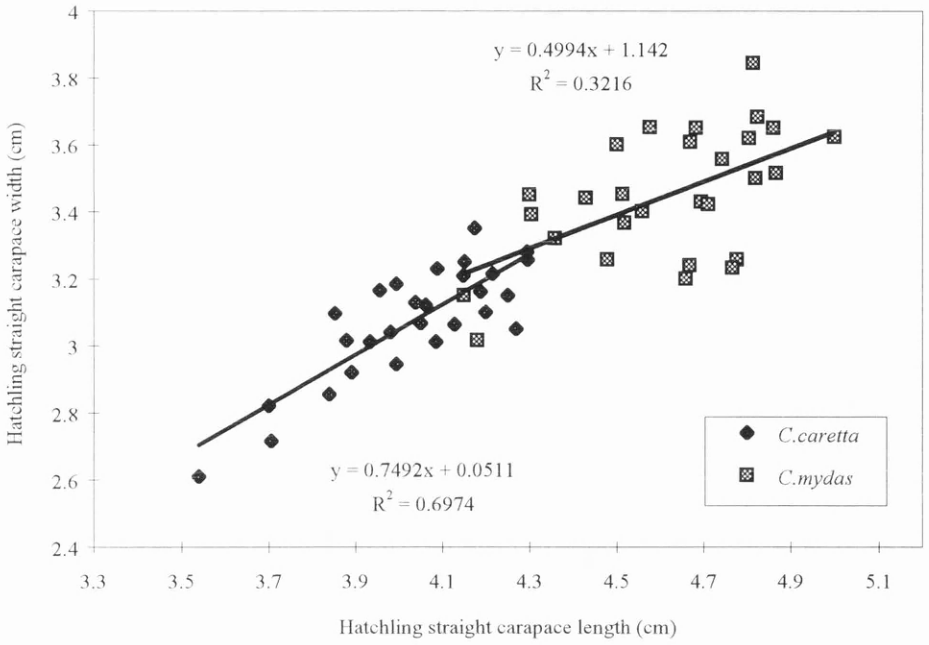


Figure 5.3.12. The relationship between straight carapace length and width of *C.mydas* and *C.caretta* hatchlings.

5.4. Discussion

In this study, larger *C.mydas* females were found to lay larger clutches at a greater depth, resulting in larger hatchlings. Similarly, larger *C.caretta* females laid larger clutches and with an increase in size, these clutches took a longer time to incubate. In addition, larger *C.caretta* females were recorded nesting earlier in the season than smaller females. Of the many other factors compared, none showed any significant relationship with female size.

There are many examples of data correlating female size to clutch size in the literature (Bjorndal & Carr 1989; Chen & Cheng 1995; Hays & Speakman 1991; Frazer & Richardson 1986; Loop *et al.* 1995). Other studies however have shown no such relationships (Ehrhart 1982; Hirth 1980). One reason for this may be the parameter used to represent female size, that is straight or curved carapace measurements. Pinckney (1990) found that in *C.caretta* nesting in South Carolina, straight carapace length was a better correlate of clutch size than curved carapace length.

No previous papers have been published reporting on the use of $PCA_{adult\ size}$ as a measure of marine turtle size. In some cases this measure may have been inappropriate. For example, in this study, *C.mydas* curved carapace length is a better correlate of nest depth. This may be due to the fact that curved carapace width, although related to length, has little to do with the depth to which a nest is dug and the measure of $PCA_{adult\ size}$ takes width into account, thus reducing its significance. However, no significant relationship was recorded between any measure of *C.caretta* size and depth of the egg chamber.

Larger (length) females migrating further up the beach before selecting a suitable nesting site, can, by virtue of their increased size dig to a greater depth in order to reach the water table. In so doing they are reducing the risk of inundation of the nest, but as a result must dig deeper nests to achieve the correct moisture levels in the surrounding sand. Smaller members of the same species will, by necessity, have to create their nest nearer to the high water mark. The same argument holds true for *C.caretta.*

Although curved carapace length was a better correlate with the depth to which a *C.mydas* female dug her nest than $PCA_{adult\ size}$, in examination of the relationship with mean clutch size, the latter was a better correlate than either length or width. However, in the case of

C.caretta, curved carapace width was the best correlate of mean clutch size. Although analysis of covariance showed no significant differences between the slopes of the relationships between curved carapace length and width of the two species, they are a different shape. *C.mydas* has a flatter shaped shell that is more circular than the humped, pear shaped carapace of *C.caretta*. Curved carapace width of *C.caretta* females may be a better indication of the capacity of the female to retain eggs prior to oviposition.

There was no linear relationship between the day of the season on which a female laid her first clutch and the size of *C.mydas* females. However, a negative relationship was recorded in *C.caretta,* with larger females being amongst the first nesters. This is not a strong relationship, as it only accounts for 8.25% of the variation. Although *C.caretta* females laying early in both the season and year laid a greater number of clutches, and larger females are recorded earlier in the season, larger *C.caretta* females do not lay a greater number of clutches. Fraser and Richardson (1986) similarly found no relationship between the number of clutches *C.caretta* females laid in a season, on Little Cumberland Island, Georgia, and their curved carapace length.

The fact that *C.mydas* females laid more clutches, on average, in 1995 than in 1993 and 1994 is most likely to be a result of improved recording. In 1995, only 3 *C.mydas* nests were not attributed to individual females (see chapter 3). Another factor that was shown to affect the number of clutches a *C.mydas* females laid in a season was the mean inter-nesting interval between oviposition. Shorter inter-nesting intervals resulted in more clutches being laid although no relationship was recorded between the length of the inter-nesting interval and the mean size of clutches laid. This relationship was also recorded for *C.mydas* nesting in Taiwan (Chen & Cheng 1995). Such a relationship was not recorded for *C.caretta.*

It is difficult to explain that with an increase in *C.caretta* adult size, an increase in the incubation period was recorded, particularly when no relationship was found between size and depth of the nest. However this could be explained by the fact that larger *C.caretta* females were recorded to lay earlier in the season and these nests would have been subjected to cooler temperatures resulting in longer incubation periods. An increase in moisture levels in a nest has been shown to increase the incubation period and the size of hatchlings produced (Gutzke *et al.* 1987; McGehee 1990). Thus, larger females might be selecting nesting sites which, although not deeper than those of smaller females, do have a

higher moisture level. Although in this study larger *C.caretta* females did not produce larger hatchlings, they did produce larger clutches. In the case of *C.mydas*, larger hatchlings emerged from these larger clutches. There is obviously a complex relationship between all of these variables which further study may reveal.

All three indices of size of nesting *C.mydas* females were related to the straight carapace length of hatchlings. $PCA_{adult\ size}$ was the best of these correlates. Chen and Cheng (1995) similarly found a positive relationship between adult and hatchling straight carapace length of *C.mydas* nesting in Taiwan. Whether this results from larger females investing more resources into offspring, laying deeper nests or larger clutches was examined. A significant relationship was recorded between hatchling size (all three indices) and clutch size. Hatchling PCA_{size} was the best correlate in this case. There was, however no such relationship between hatchling size and the depth of the egg chamber.

Information on egg size was not collected as the interference necessary to obtain data was not justifiable in this case. Handling of eggs as they are laid may introduce infections to the nest and digging up a nest to record the clutch measurements holds the same problems as transplanting a nest which has been repeatedly shown to reduce the hatching success of a nest (Whitmore & Dutton 1985). However, in a study of *C.caretta* nesting on the Greek island of Cephalonia, Hays and Speakman (1991) found no correlation between female size and egg size although a relationship between female size and clutch size was recorded. Nevertheless, by whatever means, larger *C.mydas* females produce larger hatchlings. In this study, hatchling straight carapace length and width were used which might account for adult curved carapace length being a better correlate that hatchling PCA_{size}.

In *C.caretta*, hatchling size was found to have a strong relationship with both the hatching success and hatchling emergence success of a nest. Whilst a higher hatching success was seen in nests with larger hatchlings, hatchling straight carapace length showed the strongest relationship with hatching success whilst hatchling emergence success was most highly related to hatchling PCA_{size}. Hatchling emergence success takes into account the number of hatchlings that emerged successfully from the nest not just the egg as in hatching success. So, larger *C.caretta* hatchlings appear to be both more able to emerge from the egg and sand. Thus it may be a fair assessment to suggest that larger *C.caretta*

hatchlings have a greater fitness than smaller ones. No relationship was recorded between *C.mydas* hatchling size and the success of the nest.

The depth of the egg chamber was also found to affect the success of *C.caretta* nests. Deeper nests, with respect to both the top and bottom of the egg chamber, had a higher hatching success. However, whilst nests which were deeper with respect to the top of the egg chamber had a higher hatchling emergence success, no such relationship was recorded with respect to the bottom of the egg chamber. Whilst deeper nests resulted in more hatchlings emerging from the egg, they did not result in more hatchlings emerging from the sand, possibly due to the increase in distance to the surface of the sand. Deeper nests however will probably be laid further from the waters edge and therefore may not have suffered from inundation. Deeper nests may also have a lower risk of detection by predators or being damaged due to high temperatures or human pressure from beach users.

Females may adopt different strategies depending on their size and the environmental conditions to which they are subject. To satiate predators it may be better to lay more clutches of a smaller size than fewer larger clutches. Different regimes may evolve under different conditions depending upon the pressure that exists from egg or hatchling predation. In addition, every time a female leaves the sea she too faces a threat from predation and this must also be taken into account when opting for the most efficient reproductive strategy. There also may be some constraint on the number of eggs, or weight of eggs that a female can carry without affecting her mobility in water. A large proportion of egg is fat which is less dense than water and thus holding large clutches may restrict diving ability as the distribution of the fat becomes localised within the reproductive tract.

This section has illustrated some of the clear and complex relationships that exist in determining the reproductive success of females. Many other factors, however will be acting together to influence the success of a nest and the survival of hatchlings, one of these is temperature and this is examined in the following chapter.

Chapter 6 - The Role of Temperature in the Control of the Temporal Spread of Marine Turtle Nesting and Hatching.

6.1. Introduction

Being reptiles, marine turtles are highly dependent on temperature at all stages of their life-cycle, from embryonic development and nest selection to adult growth (Mrosovsky 1994; Stoneburner & Richardson 1981). Although nesting is restricted to tropical and sub-tropical areas world-wide, marine turtles are not confined to these areas and have been found outwith these zones as far north as the waters off British Columbia and the coast of Scotland (Langton *et al.* 1996; Frair *et al.* 1972)).

The nesting season of marine turtles varies with latitude and is usually limited to the warmest months of the year. In some cases, near the equator, nesting occurs all year round (Dodd 1988). In the Mediterranean *C.mydas* has been recorded nesting between late May and early August (Coley & Smart 1992; Gerosa *et al.* 1995). In Greece, *C.caretta* nesting occurs from late May until early September (Margaritoulis & Dimopoulos 1995) whereas in Turkey nesting begins earlier at the start of May and continues until mid August (Erk'akan 1993).

The effect of an increase in sea temperature may be important in signalling females to begin their reproductive cycle, by initiating egg production, migration to the nesting ground or mating. Within the Mediterranean, the water temperature and salinity are highest in the east, owing to the enclosed nature of the sea (Weyl 1970). Data from 1994 illustrate that mean daily sea temperatures around the coast of Cyprus ranged from 20-21 °C in May, 23-24 °C in June, 26-27 °C in July and 27-28 °C in August (LEVITUS94 1996).

Many studies have examined the role of temperature on embryonic development (Bobyn & Brooks 1994; Mrosovsky 1980; Mrosovsky & Provancha 1989; Mrosovsky *et al.* 1995). The temperature at which a nest incubates has been shown to affect the duration of the incubation period, the success of the nest and the sex ratio of the hatchlings produced. Other studies have shown that the temperature range for successful hatching lies between 26°C and 32°C, with cooler temperatures within this range producing males and warmer temperatures, females (Godfrey *et al.* 1996a; Harry & Limpus 1989). The temperature at which a 1:1 sex ratio is produced has been named the pivotal temperature and the point of incubation at which the sex of the developing embryo is determined, the

critical or thermosensitive period (Mrosovsky & Pieau 1991). It is thought that this occurs sometime during the middle third of incubation (Yntema & Mrosovsky 1980).

It is crucial that the correct temperature is achieved in incubating nests which have had to be relocated or incubated artificially so that the sex ratio of the hatchlings produced is not skewed (Dutton *et al.* 1984). In addition the effects on temperature of artificial shading of nests must be considered. Whilst Mrosovsky *et al.* (1992) found there to be no difference in the sand temperatures in shaded and unshaded areas of a hawksbill nesting beach in Antigua, Mrosovsky *et al.* (1995) recorded a reduced temperature of 1-2 °C at 30 and 60 cm sand depths in nests incubating in the shade of condominiums. These nests, in turn, had longer incubation periods. However, Godfrey *et al.* (1996b) found no significant temperature differences in nests shaded by umbrellas.

Most studies have concentrated on the effect of temperature on incubating nests and the majority of these have been conducted on artificially incubated nests. In this chapter, the role of temperature on the onset of nesting and on the incubation periods of *C. mydas* and *C.caretta* nests *in situ* was examined. Although no studies have identified the pivotal temperature for nests of these species within the Mediterranean, outwith it has been shown to be approximately 29°C for both species (Mrosovsky 1988; Mrosovsky *et al.* 1984). Few temperature studies have been conducted on marine turtle nests in the Mediterranean and those that have involved interference of *in situ* or artificial incubation of nests (Billett *et al.* 1992; Kaska *et al.* 1997; Margaritoulis 1985). This study provided a rare opportunity to examine the role of temperature on the nesting and hatching of both species of turtle nesting on the same beach. The incubation periods recorded for marine turtle nests in the Mediterranean are reviewed in chapter 4 of this thesis.

6.2. Methodology

In chapters 3 and 4, baseline data are presented on the total numbers of nests recorded as laid or hatched and the methodology for this data collection is described in chapter 2. For the ease of examining the data graphically, the study period was divided into weeks as follows:

week 1 - 22nd May-28th May
week 2 - 29th May - 4th June
week 3 - 5th June -11th June
week 4 - 12th June -18th June
week 5 - 19th June -25th June
week 6 - 26th June - 2nd July
week 7 - 3rd July - 9th July
week 8 - 10th July - 16th July
week 9 - 17th July - 23rd July
week 10 - 24th July - 30th July
week 11 - 31st July - 6th August
week 12 - 7th August -13th August
week 13 - 14th August - 20th August
week 14 - 21st August - 27th August
week 15 - 28th August - 3rd September
week 16 - 4th September - 10th September
week 17 - 11th September - 17th September
week 18 - 18th September - 24th September
week 19 - 25th September - 1st October
week 20 - 2nd October - 8th October

Data on air temperature were provided by the meteorological department of Northern Cyprus. Air temperature was recorded at sea level in Girne (Kyrenia) harbour, 16 km west of this study site.

The relationship between the date upon which a nest was laid and the resultant incubation period was investigated using regression analysis for both species separately for each of the three years of this study. In all cases, possible linear and quadratic relationships were investigated. In the event of both relationships being present the significance of adding a quadratic function to the model was examined (Zar 1984). If there was no significant difference between the two models the quadratic relationship was rejected and the linear relationship accepted. If there was a significant difference between the two models the quadratic was accepted. Data on mean incubation periods for each year and species are presented in chapter 4.

Information on nest temperature for the two species was gathered in the seasons of 1995 and 1996 using 'Tinytalk data logger' temperature recorders (Orion Components Ltd., Chichester, UK). A temperature recorder was placed in the middle of each study clutch during the laying process. The recorders were programmed to record the temperature every 48 minutes over a 60 day period with a precision of $\pm$ 0.1 °C. A protective wire screen was placed over each study nest to guard against possible predation by dogs and foxes. Once hatching was completed, the data logger was retrieved and the data off-loaded to a computer.

6.3. Results

Variations in the onset and duration of nesting, and the subsequent hatching season were recorded between species and years. The dates of the start and finish of both nesting and hatching for each of the two species are given in tables 6.3.1. and 6.3.2. below. In addition the temporal spread of nesting and hatching are illustrated in figures 6.3.1. and 6.3.2. below for *C.mydas* and *C.caretta* respectively.

Nesting started markedly later in 1993 than in 1994 or 1995 for both species. Duration of the nesting season was longest for *C.mydas* in 1993 and for *C.caretta* in 1995. In 1993 and 1995 nesting by *C.caretta* preceded that of *C.mydas*. In 1994 both species started nesting on the 31st May, although many *C.caretta* false crawls had been recorded prior to this date.

	Chelonia mydas			*Caretta caretta*		
Year	**Start**	**End**	**Days**	**Start**	**End**	**Duration**
1993	16th June	25th August	70	15th June	12th August	58
1994	31st May	5th August	66	31st May	7th August	68
1995	6th June	9th August	64	31st May	19th August	80

Table 6.3.1. The nesting season and its duration, for *C.mydas* and *C.caretta* at Alagadi.

The hatching season, being heavily dependent on the nesting season and the success of nests, follows a similar pattern.

	Chelonia mydas			*Caretta caretta*		
Year	**Start**	**End**	**Days**	**Start**	**End**	**Duration**
1993	5th August	8th October	64	3rd August	8th September	36
1994	29th July	24th September	57	18th July	24th September	68
1995	4th August	25th September	52	20th July	1st October	73

Table 6.3.2. The hatching season and its duration, for *C.mydas* and *C.caretta* at Alagadi.

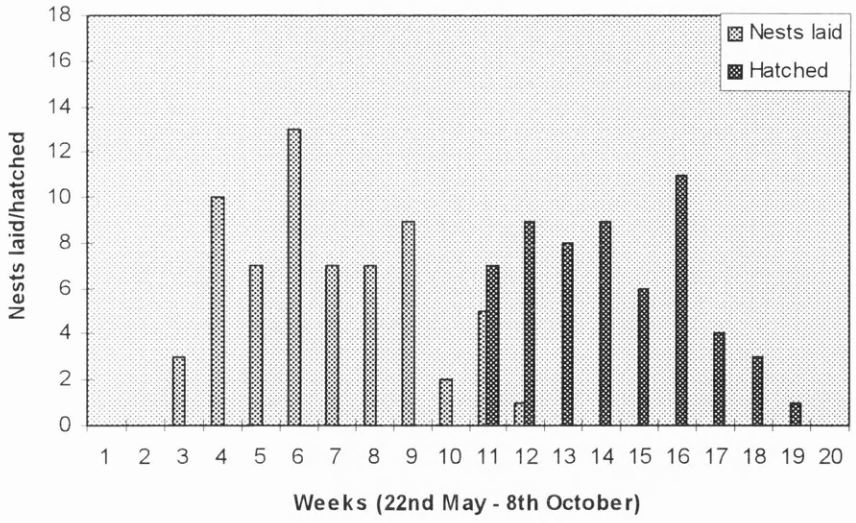


Figure 6.3.1. Temporal spread of nesting and hatching of *C.mydas,* 1995.

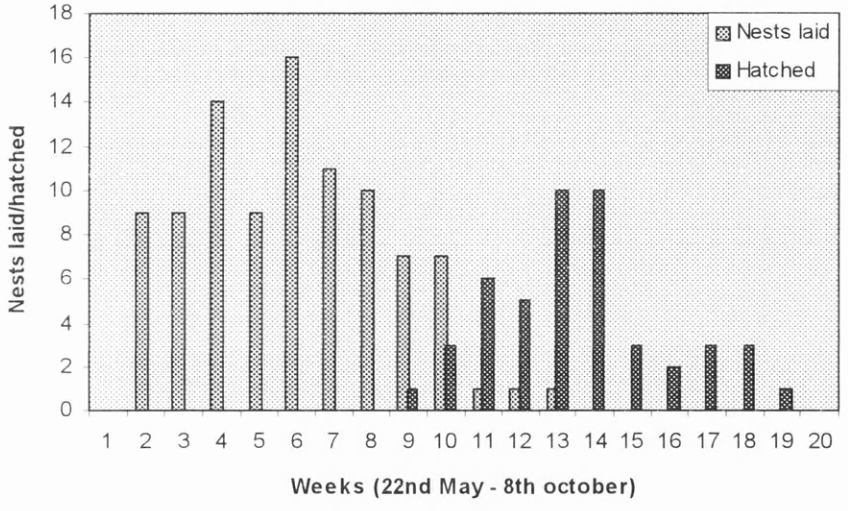


Figure 6.3.2. Temporal spread of nesting and hatching of *C.caretta,* 1995.

The relationships between daily mean air temperature and the onset of nesting for each species in each of the three years of this study are shown in figures 6.3.3.-6.3.8. In 1993, the mean daily air temperature only rose to 20°C towards the end of May, reaching 25°C a few days prior to the onset of laying. In 1994 and 1995 the temperature rose above 20°C in the middle of May and again to 25°C a few days prior to laying. Apart from at the onset of laying there does not appear to be any further relationship between air temperature and the temporal spread of nesting.

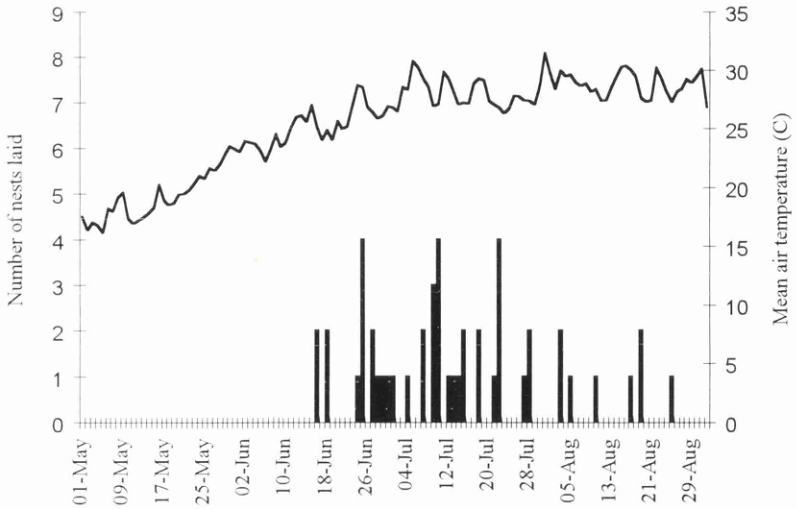


Figure 6.3.3. The temporal distribution of nesting of *C.mydas*, 1993, with mean daily air temperatures in centigrade.

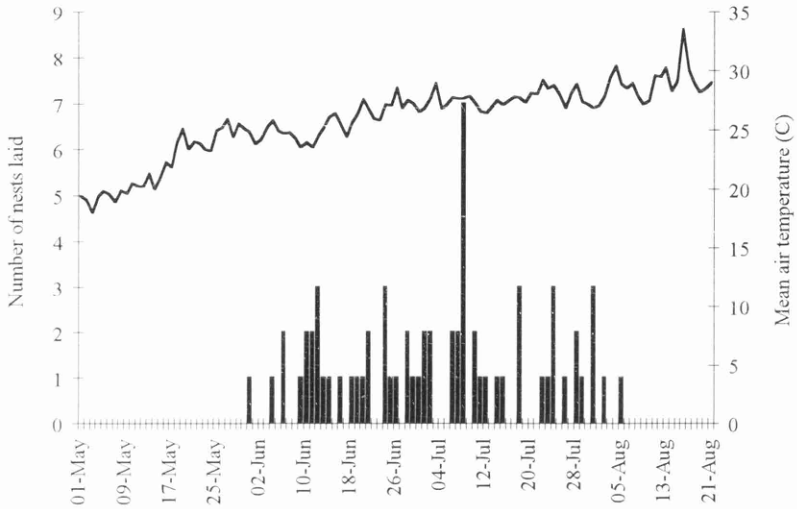


Figure 6.3.4. The temporal distribution of nesting of *C.mydas*, 1994, with mean daily air temperatures in centigrade.

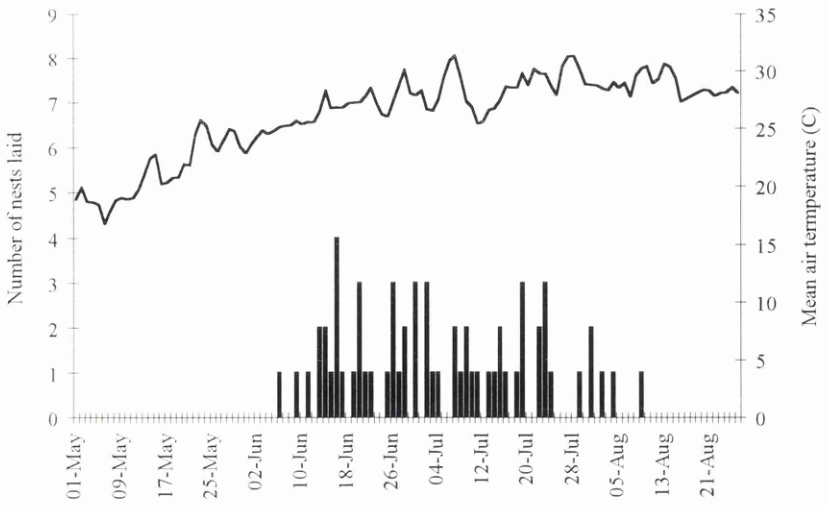


Figure 6.3.5. The temporal distribution of nesting of *C.mydas*, 1993, with mean daily air temperatures in centigrade.

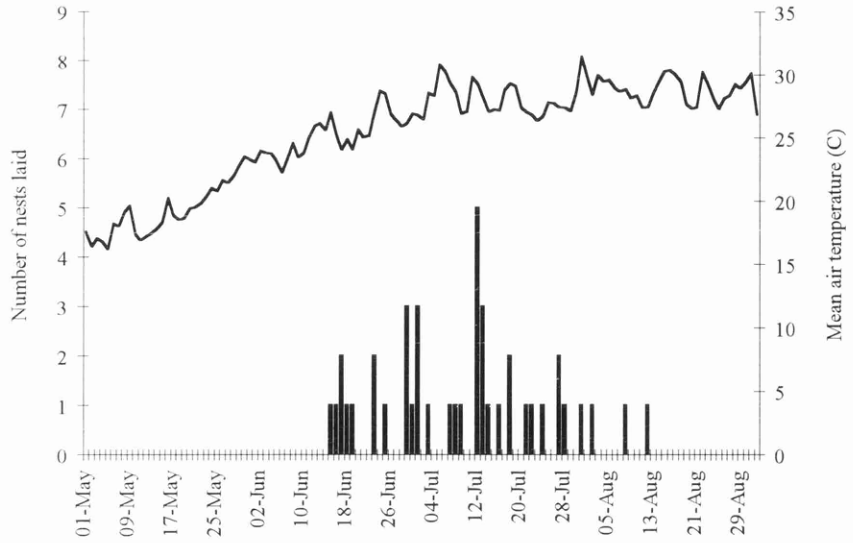


Figure 6.3.6. The temporal distribution of nesting of *C.caretta*, 1993, with mean daily air temperatures in centigrade.

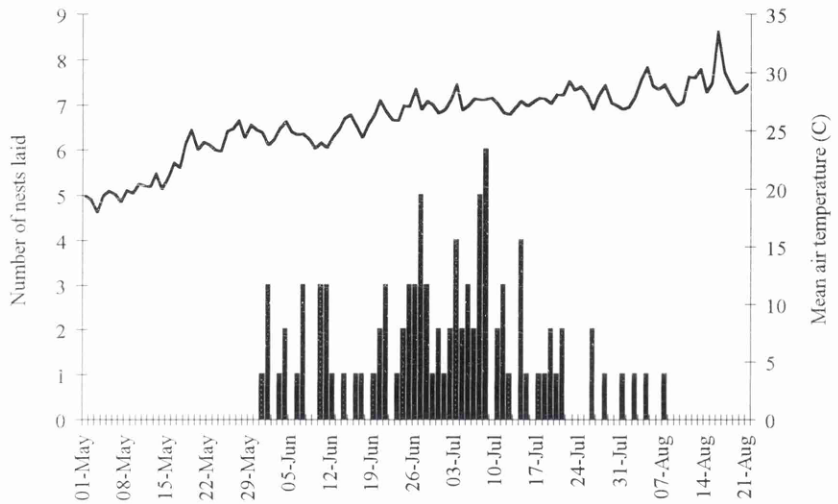


Figure 6.3.7. The temporal distribution of nesting of *C.caretta*, 1994, with mean daily air temperatures in centigrade.

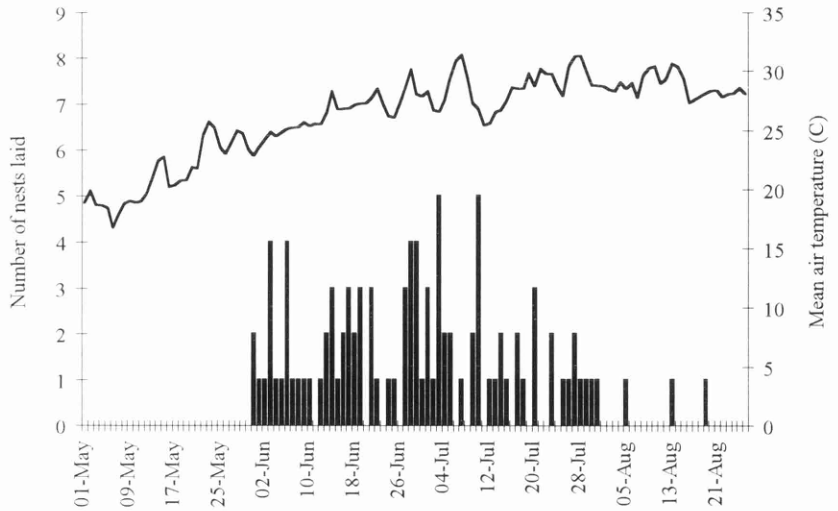


Figure 6.3.8. The temporal distribution of nesting of *C.caretta*, 1995, with mean daily air temperatures in centigrade.

In some years the relationship recorded between the date upon which a nest was laid and its resultant incubation period were linear. This was the case for *C.mydas* in 1994 (F=7.98, p=0.007, n=48) and 1995 (F=30.83, p<0.001, n=52) (figures 6.3.11 & 6.3.13.). A similar relationship was also recorded for *C.caretta* in 1994 (F= 13.06, p=0.001, n=58) (figure 6.3.12.). In all these cases relationships were negative with incubation periods decreasing as the season progressed. In 1993 no significant relationship was recorded for *C.caretta* (figure 6.3.10.) possibly due to the small sample size (F=1.3, p=0.271, n=17). However, the relationships recorded between the date of lay and incubation periods for *C.mydas* in 1993 (F=4.08, p=0.0318, n=24) and *C.caretta* in 1995 (F=11.34, p<0.000, n=40) were both quadratic. These two relationships are illustrated in figures 6.3.9. and 6.3.14. respectively. These results may be reflecting the early start or late finish of the nesting season or a combination of both factors.

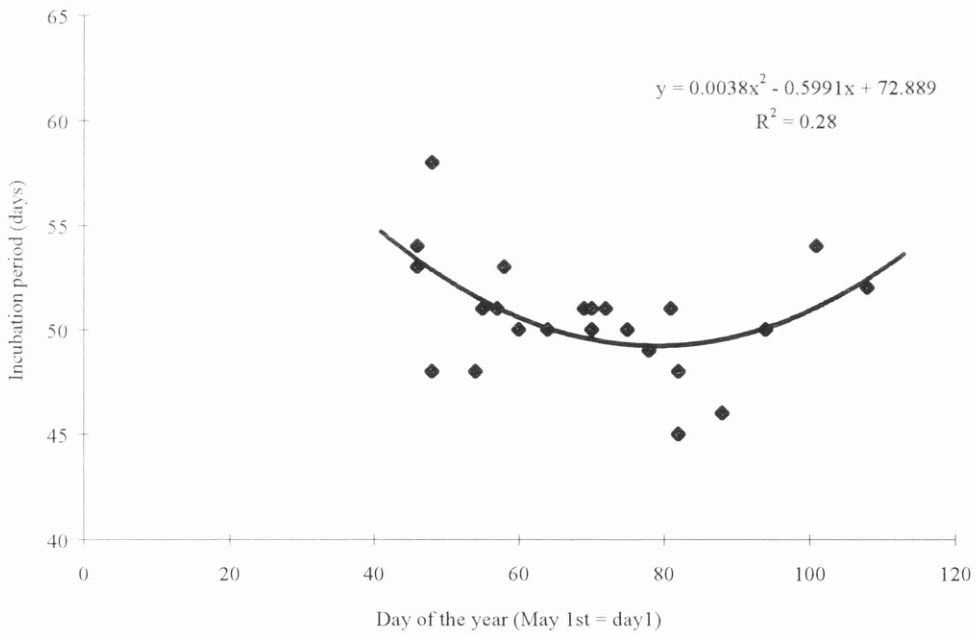


Figure 6.3.9. The quadratic relationship between the day of the year on which a nest was laid and the resultant incubation period for *C.mydas*, 1993.

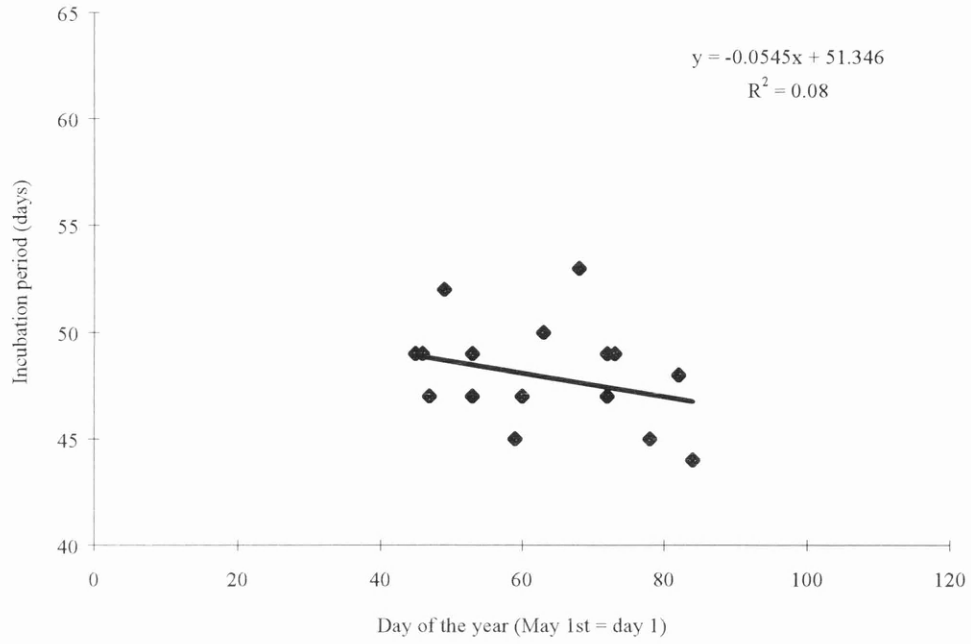


Figure 6.3.10. The linear relationship between the day of the year on which a nest was laid and the resultant incubation period for *C.caretta*, 1993.

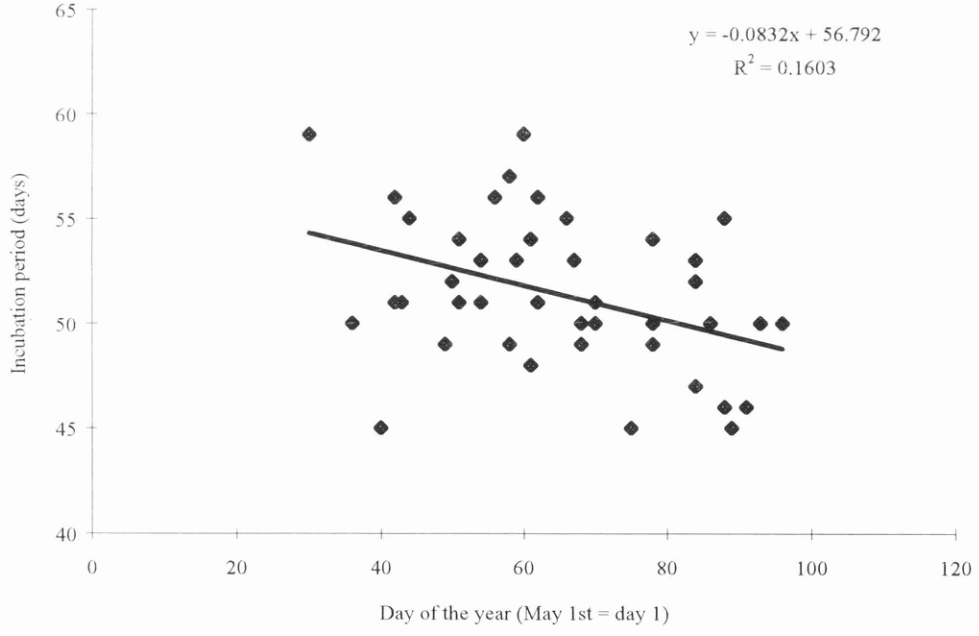


Figure 6.3.11. The linear relationship between the day of the year on which a nest was laid and the resultant incubation period for *C.mydas*, 1994.

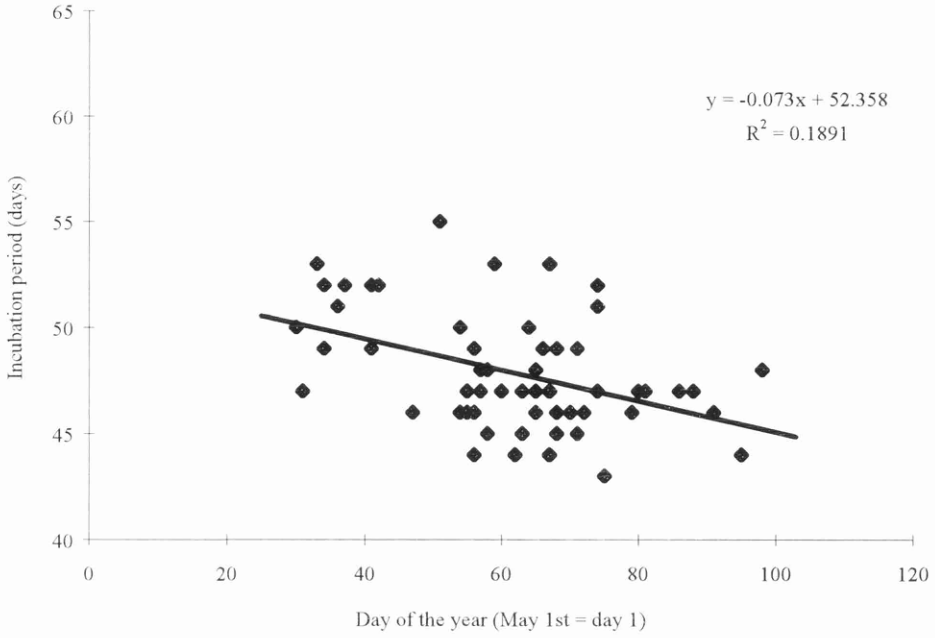


Figure 6.3.12. The linear relationship between the day of the year on which a nest was laid and the resultant incubation period for *C.caretta*, 1994.

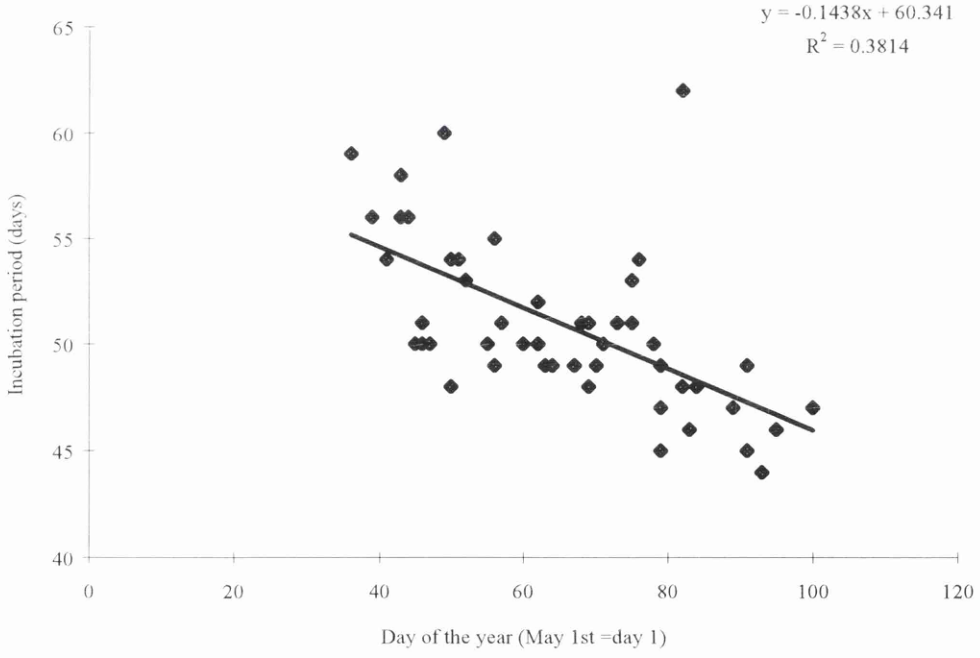


Figure 6.3.13. The linear relationship between the day of the year on which a nest was laid and the resultant incubation period for *C.mydas*, 1995.

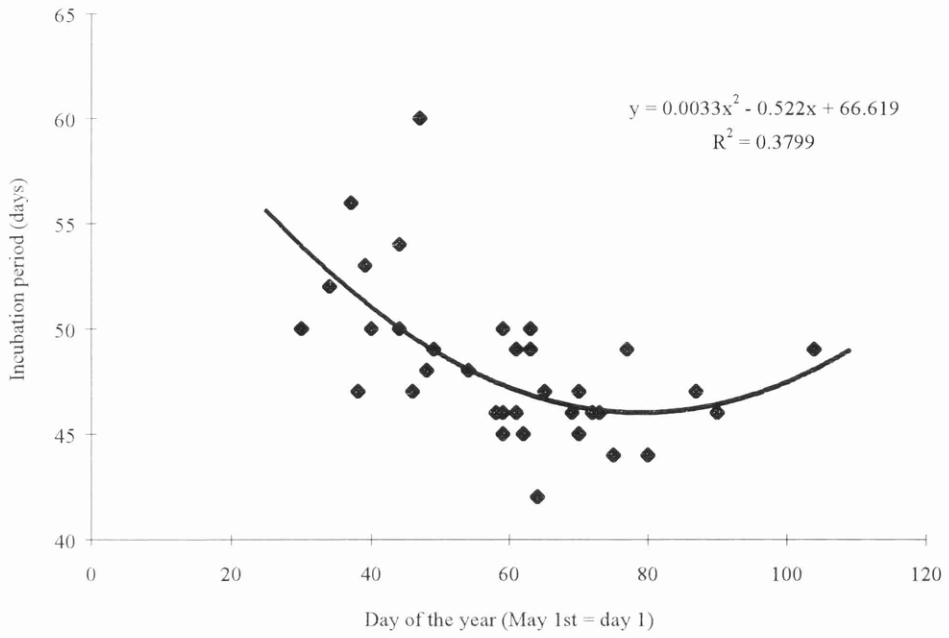


Figure 6.3.14. The quadratic relationship between the day of the year on which a nest was laid and the resultant incubation period for *C.caretta*, 1995.

Summaries of the information obtained from temperature recorders placed in three *C.mydas* nests and three *C.caretta* nests are given in tables 6.3.3. and 6.3.4. For these, the first and last day of the incubation period when the temperatures were not stable were excluded from analyses. Unlike other nests examined which were undisturbed, nest 6 (an unobserved nesting) was in part excavated so that the data logger could be placed in the clutch. The temperature variation over the incubation period is also illustrated for each of the six nests in figures 6.3.15-21.

Nest details	*Nest 1*	*Nest 2*	*Nest 3*
Date of lay	23/6/95	26/6/95	29/6/96
Temperature range °C	28.5 - 33	29.4 - 32.1	28.5 - 32.5
Mean daily temp °C	30.6 (±0.22, n=51)	30.8 (±0.14, n=48)	30.7 (±0.16, n=49)
Mean daily range °C	0.14 (± 0.03, n=51)	0.07 (± 0.03 n=48)	0.09 (±0.03, n=49)
Daily temp range °C	0 - 0.5	0 - 0.5	0 - 0.5
Incubation period	52 days	49 days	50 days
Clutch size	71	70	98
Hatching success %	73	97	96
Depth of clutch (cm)	61 - 89	69 - 80	67 - 90

Table 6.3.3. Nest details of *C.mydas* nests in which temperature recorders were placed, with standard errors and sample sizes where appropriate.

The overall range in temperature found in *C.mydas* nests was 28.5 - 33°C. All three *C.mydas* nests were laid towards the start of the nesting season and a steady increase can be seen in the temperatures recorded within the nest as the season progressed, levelling out in early August (see figures 6.3.15.-6.3.17.). The maximum daily range, in all cases, was 0.5 °C. Diurnal fluctuations are rarely seen, most changes are a result of general trends.

Figure 6.3.18. presents the last 36 hours of the incubation period of *C.mydas* nest 3. Fluctuations of this nature were not seen in any other nests. Excavation of the nest contents occurred at approximately 7 am on the morning of the 18/19th August, this resulted in the marked decrease in temperature seen.

Nest details	*Nest 4*	*Nest 5*	*Nest 6*
Date of lay	13/6/95	17/6/95	13/8/95
Temperature range °C	28.9 - 32.5	28.1 - 33	28.9 32.1
Mean daily temp °C	30.8 (±0.19, n=47)	30.7 (±0.22, n=48)	30.6 (±0.12, n=46)
Mean daily range °C	0.432 (±0.03, n=47)	0.302 (±0.03, n=48)	0.65 (± 0.04, n=46)
Daily temp range °C	0 - 0.9	0.4 - 0.9	0.4 - 1
Incubation period	49 days	48 days	49 days
Clutch size	59	94	41
Hatching success %	81.4	96	44
Depth of clutch (cm)	33 - 53	47 - 70	36 - 48

Table 6.3.4. Nest details of *C.caretta* nests in which temperature recorders were placed, with standard errors and sample sizes where appropriate.

In the three *C.caretta* nests, temperatures ranged from 28.1 - 33°C, however in all cases the daily variation was higher than in *C.mydas* nests, ranging from 0-1°C. This is reflected in the mean daily range in temperatures which are much larger than those of *C.mydas* nests. When examining these data closely (figures 6.3.19. - 6.3.21.), a marked diurnal effect can be seen on most days. Nest 6, (figure 6.3.21.) was the last nest laid in 1995 and a steady decline in the temperature within the nest is seen as the period progresses. In nests 4 and 5, laid in 1996, (figures 6.3.19 and 6.3.20) a steady increase in temperature is observed with these nests laid toward the beginning of the nesting season. At the end of the incubation period of nest 5 large fluctuations in temperature are evident, these are due to the fact that the nest was partially excavated on the 4th August having hatched in part. The remaining contents were then covered over, with the final hatching and excavation occurring on the 6th August.

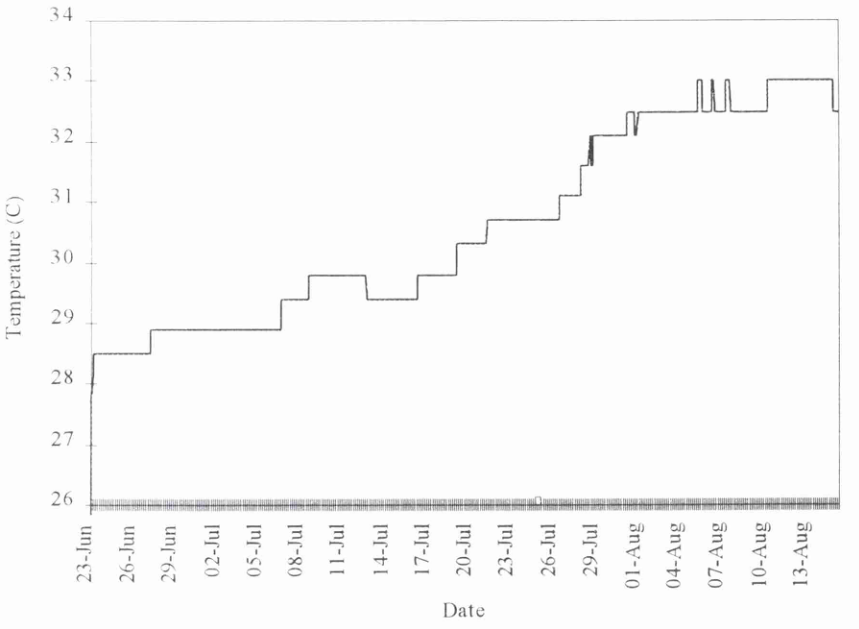


Figure 6.3.15. Nest 1 - Temperature profile of a *Chelonia mydas* nest laid on the 23rd of June 1995 and hatched on the night of the 14th/15th of August 1995.

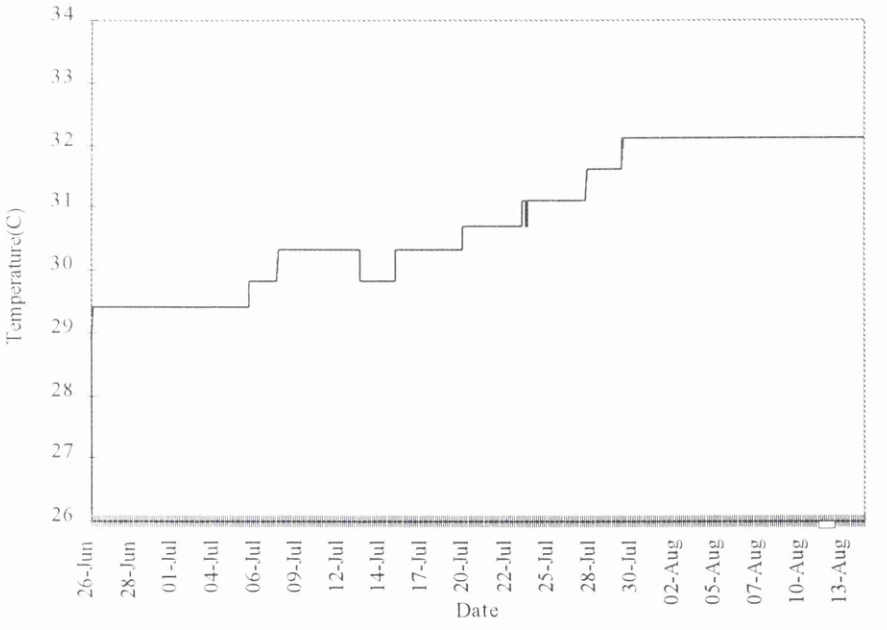


Figure 6.3.16. Nest 2 - Temperature profile of a *Chelonia mydas* nest laid on the 26th of June 1995 and hatched on the night of the 14th/15th of August 1995.

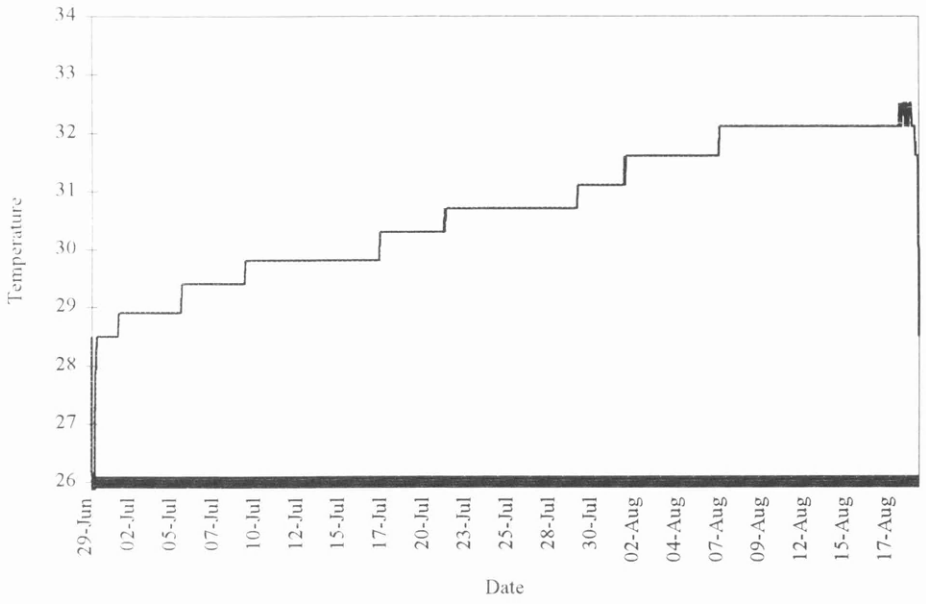


Figure 6.3.17. Nest 3 - *C.mydas* nest laid on the 29th of June 1996 and hatched on the 18/19th of August 1996.

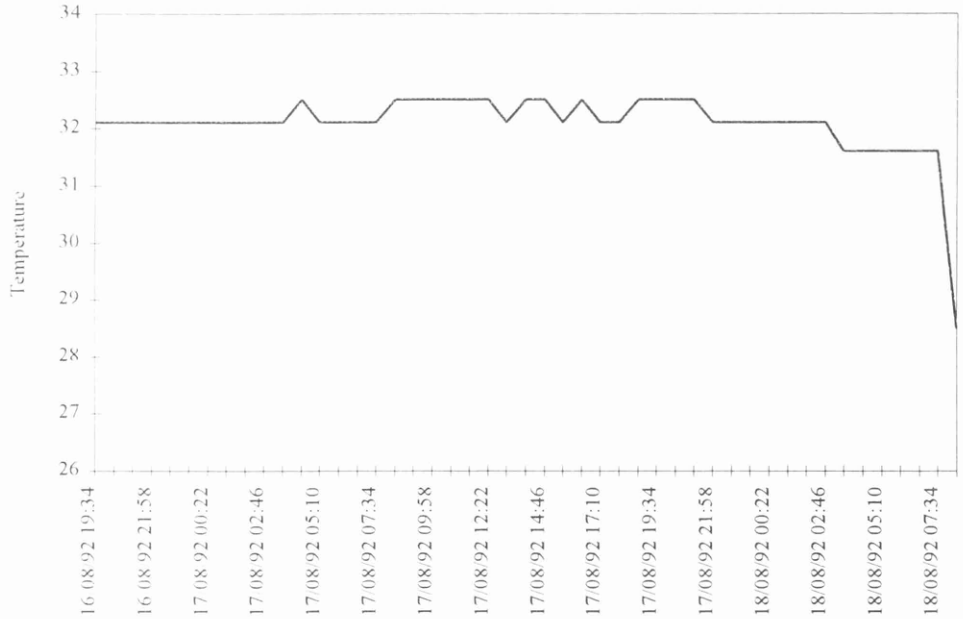


Figure 6.3.18. The period of hatching of *C.mydas* nest 3.

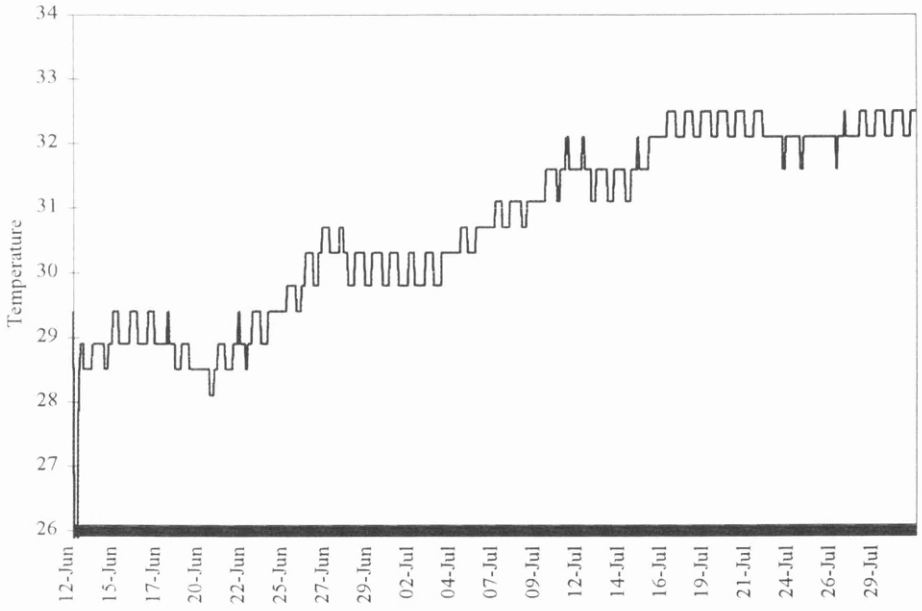


Figure 6.3.19. Nest 4 - *C.caretta* nest laid on the 13th of June 1996 and hatched on the 1st/2nd of August 1996.

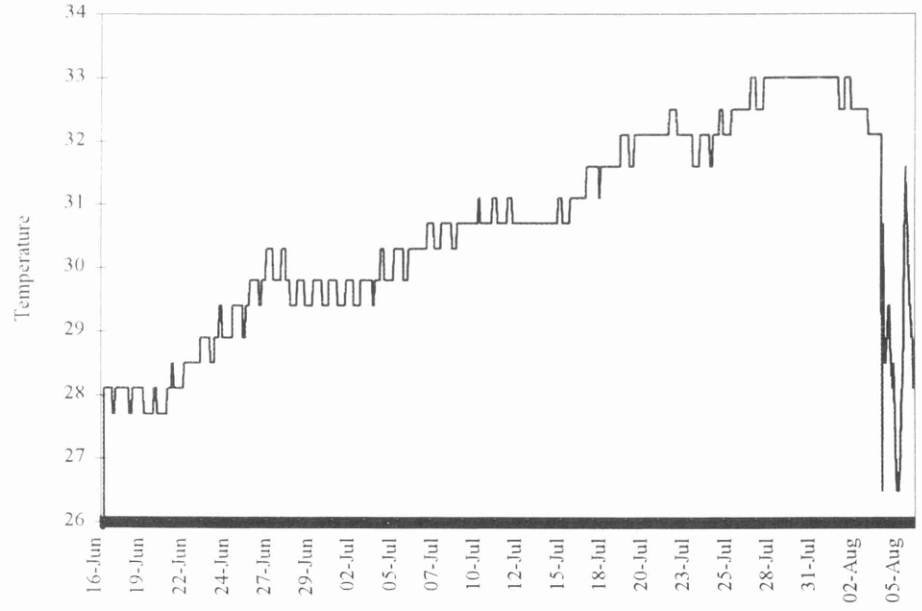


Figure 6.3.20. Nest 5 - *C.caretta* nest laid on the 17th of June 1996 and hatched on the 4th and 6th of August 1996.

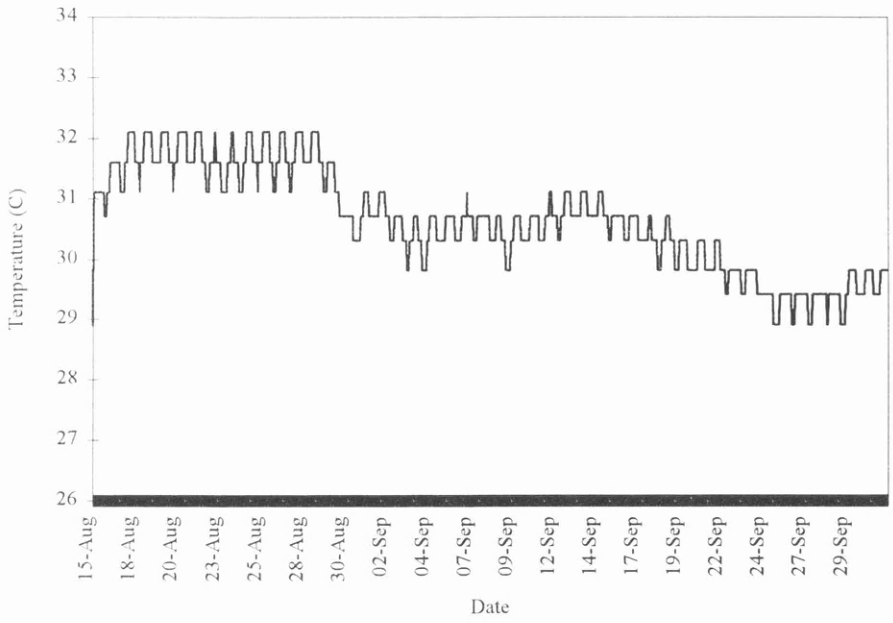


Figure 6.3.21. Nest 6 - Temperature profile of a *Caretta caretta* nest laid on the 13th of August 1995, and hatched on the night of the 1st/2nd of October 1995.

6.4. Discussion

The temporal spread of nesting of *C.mydas* and *C.caretta* are in broad agreement with previously published reports of nesting on the Mediterranean islands (Demetropoulos & Hadjichristophorou 1989; Margaritoulis 1989) and southern Turkey (Coley & Smart 1992; Peters & Verhoeven 1992; van Piggelen 1993). It is apparent from figures 6.3.3-6.3.8 that nesting began when mean air temperature reached approximately 25°C. However it may, in fact, be the rise in temperature some weeks previously that is actually the trigger, thus allowing for the physiological preparation and the production of a clutch prior to laying. This may be the reason for the slightly later start to the nesting season recorded in 1993. In reality females, and indeed males, may be dependent on temperature cues at feeding sites prior to migrating to mating or nesting sites. Of course other factors may be contributing to this delay such as food availability and the temperatures experienced since their last breeding season or lunar cycles (Frazer 1983). However it is highly probable that temperature is important in triggering the onset of nesting to ensure that nests are incubated within a temperature range that allows successful embryonic development.

Mrosovsky and Pritchard (1971) reported that the body temperature of *D.coriacea* females at nesting was 3°C lower than the sea temperature. Although the data presented are for air temperature, a strong correlation with sea temperature is expected and hence a relationship between air temperature and female body temperature. The slight delay in the appearance of *C.mydas* may be a time lag effect due to their larger mass taking longer to react to an increase in ambient temperature or possibly their feeding grounds are further away than those of *C.caretta.*

It is not, however clear what factors may be responsible for the end of the nesting season. There is no apparent temperature change associated with this period. In fact nesting begins to tail off at the height of the summer. Perhaps this is a cue in itself and signals the fact that the temperature will soon be decreasing and as a result become too cold for successful incubation. However, the end of nesting may simply be due to the depletion of fat reserves or mature follicles for egg production. It is likely that these two factors are inter-linked in some way.

It should be pointed out that during the summer months in Cyprus it is very unusual to experience either rain or cloud cover. At either end of the season, however, high winds can occur which may result in nest cooling and possible over-wash or erosion of nests due to wave action.

Many studies have questioned how a rise in temperature due to global warming will affect such temperature dependent species (Mrosovsky & Provancha 1989; Mrosovsky & Provancha 1992). However, as Mrosovsky *et al.* (1984) point out, those populations or individuals that have a low site fidelity and season fidelity will stand a better chance of reproducing successfully. If the start of the nesting season is dependent on the ambient temperature reaching a certain level then surely, if global warming does occur, females may shift their nesting season or location, effectively nesting in the same temperature range as before. There is no reason to suppose that they will continue to nest during the same calendar months subjecting themselves and their offspring to excessively high temperatures.

The relationships recorded in this study between the date of lay and the incubation period of nests can be explained with reference to an increase in temperature which in turn decreases the resultant incubation periods of nests (Mrosovsky *et al.* 1995). In 1993, the nesting season began approximately two weeks later than in 1994 and 1995 and consequently nesting of *C.mydas,* continued until late August. Nests laid toward the end of the season will then have been subject to lower temperatures with some nests incubating through until October. Thus, an increase in incubation periods, of nests laid toward the end of the nesting season was experienced and a quadratic relationship recorded. In 1993, neither a linear nor quadratic relationship was recorded for *C.caretta,* possibly as nesting ended nearly two weeks earlier than *C.mydas* that year. However, in 1995, a quadratic relationship was recorded between the date of lay and the incubation period of *C.caretta* nests. Although an early start to the nesting season was recorded in this year study, nesting of *C.caretta* females continued until after mid August. Pinckney (1990) recorded a similar quadratic relationship for *C.caretta* nesting in South Carolina. In 1994, for both species, this relationship was linear, possibly due to the early end of the nesting season and the high temperatures which prevailed in that particular year.

The present investigation has shown that both *C.mydas* and *C.caretta* nests incubate within the temperature range 28-33°C in Cyprus. If the pivotal temperature for these two species is

29°C, the sex ratio of turtles hatched in Cyprus will be skewed towards a greater number of females. It might be expected that nests laid at the start or end of the season would be subject to cooler temperatures and may hatch more males. This is considered unlikely since the greater part of the incubation period, particularly the middle third during which the sex is thought to be determined, is spent at temperatures above 29 °C. and even the last *C.caretta* nest laid in 1995 incubated constantly above 29 °C.

Similar results were recorded by Kaska *et al.* (1997) in nests of both species on the west coast of Cyprus, although temperatures within nests ranged more widely between 24.9-34.5°C. However, as is the case for many such studies, nests had to be excavated in order to place temperature data loggers into them and were thus disturbed. The greater range in temperatures recorded by Kaska *et al.* (1997) may be the result of earlier nests in a season being recorded or it is possible that the authors included data for the period before the data loggers and recently dug sand had stabilised. Alternatively this variation could be a result of the varying beach conditions at this close but different study site. However, as Kaska *et al.* (1997) point out, during the middle third of incubation all nests examined were incubating at a temperature above 29°C.

A second important difference recorded in this study between the two species is the diurnal variation in temperature regularly seen in nests of *C.caretta*. This was also reported by Kaska *et al.* (1997) in *C.caretta* nests but not in nests of *C.mydas*. This is undoubtedly due to their shallower nature which may make these nests more unstable and vulnerable to changes in environmental conditions.

All previous studies on the effects of incubation temperature on the nests of marine turtles have been conducted on artificially incubating nests at constant temperatures. No studies have attempted to mimic the temperature fluctuations recorded *in situ*. The variation that has been recorded in this investigation throughout the incubation periods may influence the course of the development of the embryos and has largely been ignored to date.

The information gained from this study suggests that unless the pivotal temperature is different in the Mediterranean than elsewhere, the sex ratio of turtles hatched in Cyprus will be skewed towards a greater number of females. It is necessary however, to examine more nests and the variation that exists both temporally and spatially before any conclusions are

made, as thermal variation has been reported on beaches in the same region of varying substrate types (Hays *et al.* 1995). Nests in Cyprus have the shortest incubation periods recorded in the Mediterranean (chapter 4). It is tempting to hypothesise that, whilst a higher female sex ratio may result in Cyprus, in other parts of the Mediterranean this may be balanced by lower incubation temperatures resulting in a higher proportion of males. However, recent studies suggest that little genetic mixing occurs between marine turtles nesting at different sites in the Mediterranean (Schroth *et al.* 1996). However, male mediated gene flow has not been documented in this region. Similar results have been recorded for *C.mydas* nesting in Cost Rica (Peare 1994). Thus, each individual population may need to produce enough males and females to ensure adequate reproductive potential and genetic variation within that population.

The fluctuations in temperature recorded in *C.mydas* nest 3 towards the end of incubation (figure 6.3.18.) may be a result of an increase in metabolic heat as the hatchlings begin to break out of their shells and move through the sand. The actual movement of the hatchlings up through the sand column may result in a cooling in the nest temperature as sand and air from the surface filters down (Gyuris 1993).

Given that an increase in temperature has been shown to decrease the length of the incubation period of marine turtle nests, it is surprising to note the shorter incubation period of *C.caretta* nests in light of the similar incubation temperatures recorded in nests of both species in this study. The reason for this difference is not clear. In studies of conditions within the nest, an increase in moisture levels has been shown to increase the duration of incubation periods (McGehee 1990).

In a controversial article Vogt (1994) suggested that as a tool to conservation it made sense to incubate marine turtle nests above the pivotal temperature as this both reduces the chances of intersexes being produced and increases the number of females which, as he points out, are the limiting factor in the reproductive potential of a population. However, Lovich (1996) comments that whilst skewed sex ratios are frequently recorded, very little is known of the adult sex ratio of marine turtles and the survivorship of the two sexes to adulthood.

One other point that is largely ignored is that, as is the case for many other species exhibiting a form of environmental sex determination (ESD), there must have been some advantage to having this method of sex determination when it originally evolved. (Ewert & Nelson 1991).

However this does not necessarily mean that that advantage still exists. It may be that in some years the conditions are such that it makes sense to have female offspring and in other years males or larger females rather than small males.

Thus far in this thesis, the depth of the nest together with the day of the season on which a clutch is laid all appear to influence the incubation period and as a consequence exert an effect on the sex ratio of hatchlings. It is feasible that turtles adopt different strategies in different years to overcome the problems experienced. By manipulating the sex ratio in artificial nests the reasons for these variations are being ignored. It is thus important to attempt to find methods of incubating nests successfully *in situ*. Intervention and manipulation of sex ratios may become necessary if situations become critical and great care will then be required.

Chapter 7 - Nesting Behaviour of *Chelonia mydas* and *Caretta caretta* and the Resultant Success of Nests.

7.1. Introduction

The stages of nesting behaviour of marine turtles are essentially the same in all species although within each stage inter-species variation occurs (Hendrickson 1982). The process is usually categorised into seven events from leaving until re-entering the sea. It has been suggested that external stimuli trigger the switch from one stage of the process to another (Hailman & Elowson 1992) and that the nesting process is pre-determined with all females, even those injured and unable to carry out each action, attempting to follow the set pattern (Margaritoulis 1985).

Although numerous studies have been carried out on the subject of marine turtle nesting activity, and many anecdotal accounts of nesting behaviour made, few are precise and little attempt has been made to explore the relationship between nesting behaviour and the resultant success of nests (Carr 1982; Dodd 1988; Ehrhart 1982; Hailman & Elowson 1992; Hays & Speakman 1991; Hendrickson 1982; Johnson *et al.* 1996; Margaritoulis 1985).

In the light of the increase in ecotourism and interest in marine turtles, Johnson *et al.* (1996) examined the effects of 'turtle watches' on *C.caretta* nesting in Florida. They found that females spent significantly less time covering their nest when observed by a 'turtle watch' group than if observed by only a single investigator. These females also took a less direct path to the sea on their return crawl. However, no effect of these behavioural changes on hatchling production was found.

In a study in Costa Rica, Campbell (1994) observed changes in the nesting behaviours of *C.mydas* females, when exposed to flash photography during laying and covering of their clutch. Females subject to the flashing spent less time covering their egg chamber than those that were not. The time spent depositing the clutch was not significantly different, although in both groups was correlated to the size of the clutch. Despite the fact that no effects on the success of the nests were studied, it was recommended that flash photography of nesting adults should be prohibited.

Although nearly all marine turtle studies involve marking adults with tags of some type, little effort has been made to assess the effect of tagging females, on their subsequent nesting behaviour and the success of the nest. Tags are retained with varying success, depending on the type and position (Eckert & Eckert 1989; Limpus 1992), however, in most instances females require new tags each year in which they nest, and are usually double tagged to reduce the effect of tag loss. Whether tagging females is detrimental has not been established, nowadays advanced techniques, such as passive integrated transponders (PIT tags), are being used (McDonald & Dutton 1994; Parmenter 1993) and are proving much more successful than conventional tagging methods. The longevity of these chips could reduce human interference of nesting females.

In this chapter, the observed sequences of behaviour are described for *C.mydas* and *C.caretta* nesting in Northern Cyprus. Comparison is made with three parameters which are used as indices of reproductive output, these are clutch size, hatching success and hatchling emergence success. The possibility of any effects of behaviour on these parameters is investigated. In addition, female size is examined in relation to reproductive output and behaviour. Lastly, the duration of the last three stages of nesting are examined to compare the effect of tagging females on their subsequent behaviour and success of nests.

7.2. Methodology

Data were collected during night time beach surveys throughout the nesting season and dawn surveys were conducted during the hatching period, according to the protocol given in chapter 2. When a female was observed, the time was noted and her stage of nesting classified into one of the seven categories below;

1) 'Ascent'
2) 'Digging body pit'
3) 'Digging egg chamber'
4) 'Laying'
5) 'Covering egg chamber'
6) 'Covering body pit'
7) 'Descent.'

The duration of each stage was recorded. In this chapter, times at which females emerged are given, irrespective of whether they subsequently nested. Analysis of behaviour however, only included occasions where successful nesting was observed.

Data were analysed using a one way ANOVA in order to test if there were any differences in the behaviours recorded in any of the three years of the study. In addition, all seven stages of behaviour were compared to one another by way of a correlation matrix in order to determine if significant relationships were present or if the duration of the stages were independent of one another.

Descriptive data for female curved carapace length and width, clutch size, hatching success and hatchling emergence success are given in the results section of chapter 4. Principle Components Analysis was again used, to represent female size. As in previous chapters this will be referred to as $PCA_{\text{adult size.}}$ Regression analysis was performed to establish if any of the seven behavioural stages were related to female size (length, width or $PCA_{\text{adult size}}$) or the three parameters of reproductive output (clutch size, hatching success and hatchling emergence success). Additionally the relationships that exist (presented in chapter 5) between the four parameters of female size and clutch size, hatching success and hatchling emergence success are summarised herein. If data were not normally distributed and normality could not be obtained through transformation, Spearmans correlation was used to test for relationships between the above variables.

The relationship between hatching success and hatchling emergence success was not investigated as these two variables are not independent. Hatching success is the percentage of eggs in a clutch which hatch, whereas hatchling emergence success is the percentage of hatchlings which successfully emerge from the sand. Thus, they are both calculated from the same data set. A further comparison was made in which the time spent at each stage of the nesting behaviour in hatched nests was compared with that of unhatched nests. For this a student t-test was used.

All females were tagged twice, once on each fore-flipper, to decrease the effect of tag loss. If a female lost one tag, or it became unreadable, within or between nesting seasons, a new one was attached. An effort was also made to assess whether tagging of females had any effect on their subsequent behaviour, with respect to nesting. In this study, females were tagged immediately after laying was complete, thus any behavioural effect from tagging would only be expected in the last three stages of nesting (covering the egg chamber, covering the body pit and the descent to the water). For this analysis, females were separated into three groups, those that were tagged once, tagged twice and those that were not tagged at the time of observation (i.e. already had tags in place having previously nested). A one way ANOVA was used to test the whether the duration of each of the three behaviours or the hatching success of nests differed in each of the three categories. The behavioural variations that exist were also examined with respect to clutch order. All statistics were analysed using MINITAB.

7.3. Results

7.3.1. Time of emergence of nesting females

The times at which females emerged onto the beach at night were examined collectively for the data recorded in 1993-1995. These were divided into the three classified activities of nest, false crawl attempt and false crawl U-turn, and are illustrated in figure 7.3.1. for *C.mydas* and 7.3.2. for *C.caretta*.

The peak time of emergence of *C.mydas* females, going on to nest successfully was between 00:01-01:00 with the majority of nesting occurring between 22:01 and 03:00. *C.caretta* females emergence times were similarly spread between 22:01 and 03:00, with peak nesting occurring between 23:01 and 00:00. False crawl attempts in both species peaked between the hours of 23:01 and 00:00. False crawl U-turns similarly occur most frequently between the hours of 23:01 and 00:00 for both species. No individuals emerged on Alagadi beach, during daylight, although it was not uncommon for a female to be on the beach at dawn, completing her nesting process. In Cyprus dusk occurs between 19.00 and 22:30 hours and dawn between 04:00 and 06:00 depending on the date.

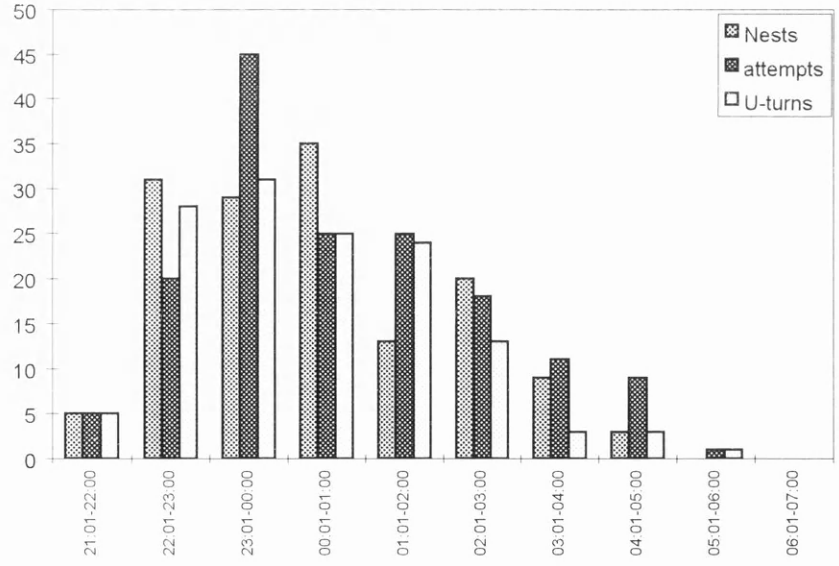


Figure 7.3.1. Time of emergence of *C.mydas* females carrying out the three activity types.

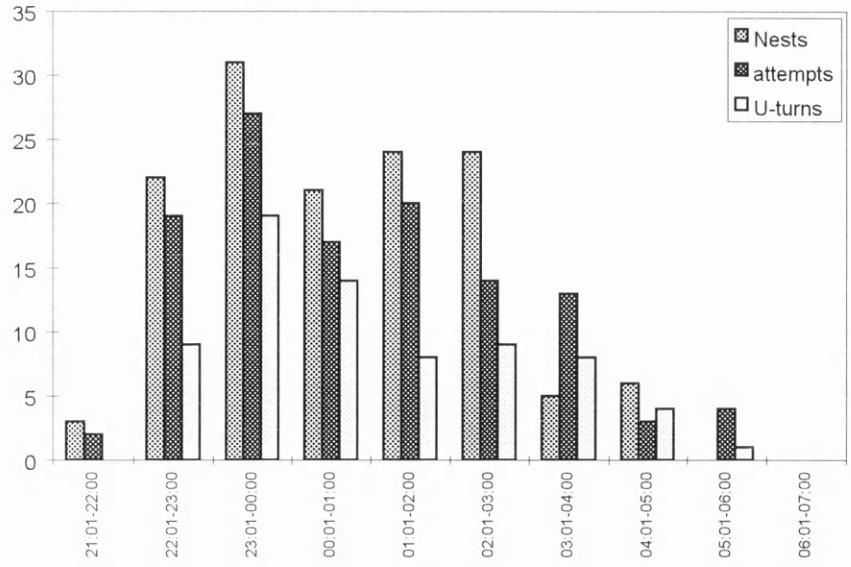


Figure 7.3.2. Time of emergence of *C.caretta* females carrying out the three activity types.

7.3.2 .Description of nesting behaviour

There are many differences in the nesting behaviour of the two species. Firstly, *C.mydas* moves her flippers in unison, leaving a symmetric track whereas *C.caretta* moves on land as if walking, moving left and right flippers alternately, creating an asymmetric crawl track. If she finds a suitable nesting site the female begins to dig a body pit using all four flippers. *C.mydas* digs a body pit, often greater than her own depth, whereas the body pit of *C.caretta* is usually very shallow. Whether due to site unsuitability, disturbance or physiological condition the female, of either species, may return to the sea without any digging attempt or leave after starting to dig a nest.

After completion of the body pit, the female of both species begins to dig an egg chamber, a tubular hole, perpendicular to the surface. For this she uses only her hind flippers. During the digging of the egg chamber, she holds one flipper on the rim of the hole, apparently providing support and preventing any sand falling in. The other flipper is used to scrape the sand, scoop it out of the chamber and throw it out behind the turtle. As this occurs, the supporting flipper flicks away sand in its vicinity and the process is repeated, alternating the flippers. As the female reaches a greater depth she may rise up on her fore flippers to gain added depth with her hind flippers.

During oviposition, *C.mydas* females were observed to hold at least one hind flipper inside the egg chamber possibly to prevent too much sand entering. *C.caretta* however, positioned both hind flippers on the sand around the egg chamber pushing down on the sand and raising them as she deposited her eggs. At any point during the process, females may abandon the nesting attempt, possibly due to related to factors such as the site being too rocky, the sand continually collapsing, or being saturated with water. However once she has completed her egg chamber and started laying her eggs it is rare for her to abandon the site.

Once the female has finished laying her eggs, she begins to cover them with her hind flippers, alternating them to scoop up sand from beside her and pat it down on top of the eggs. She then begins to cover over her nest and body pit by spraying sand backwards with all four flippers. In *C.mydas,* the latter is pronounced and the female digs her way forward, usually moving more than a whole body length, leaving a large pit. *C.caretta* throws sand back until a small pile of sand lies over the site of the eggs. Whilst covering over her nest, the female usually orientates herself so that when she vacates the site she is facing towards the sea, leaving a U shaped spray of sand behind her.

7.3.3. Unusual Observations

After the observation of some 1000 nesting activities over this study period, some anecdotal observations are described at this stage to highlight the vagaries that exist within the 'stereotypic' behaviour patterns.

On two occasions, *C.mydas* females successfully nested and then moved up the beach, rather than returning to the water. These individuals then attempted to dig a further nest. There was no additional clutch laid in either case. On two other separate occasions *C.mydas* females were observed to excavate other females nests whilst digging their own, subsequently abandoning their nesting attempt. In addition, one *C.mydas* successfully laid her clutch and was then observed to uncover it whilst covering over her body pit.

On four separate occasions observations of mating green turtles were made. On one occasion the pair were in the sea, approximately 5 metres from the shore, whilst on another, a mating pair appeared to have been washed onto the beach in stormy weather. In both cases the pair were observed to remain attached for approximately 5 minutes. However, on the other two occasions, the female crawled onto the beach with the male firmly attached.

After ascending approximately 5-10 m up the beach, the male in each case detached, apparently disorientated, and returned to the water. Both females went on to attempt nesting, one laying a clutch which successfully hatched. In this final instance, the male was measured. Curved carapace length was 85 cm. No cases of these phenomena have been reported in the literature.

7.3.4. Duration of behavioural stages

The average time spent during each stage of nesting for *C.mydas* and *C.caretta* are given in table 7.3.1. No significant differences were recorded in the duration of behaviours in the three years of this study. *C.mydas* spent longer than *C.caretta* at every stage of the nesting process, particularly during the covering of the body pit which takes almost five times longer in *C.mydas*. *C.mydas* females spent, between 94 and 276 minutes executing the nesting process, whereas *C.caretta* could complete nesting between 36 and 101 minutes.

	Chelonia mydas					*Caretta caretta*				
Nesting Behaviour	*N*	*Mean*	*S.E.*	*Min.*	*Max.*	*N*	*Mean*	*S.E.*	*Min.*	*Max.*
Ascent	29	19.93	4.7	3	112	12	9.08	1.89	2	22
Dig body pit	47	21.4	1.39	5	50	12	7.2	0.76	4	10
Dig egg chamber	83	26.2	1.17	5	67	41	14.5	0.99	5	30
Lay eggs	102	14.5	0.65	3	40	93	11.8	0.48	1	25
Cover egg chamber	111	8.7	0.4	1	27	108	7.2	0.48	1	48
Cover body pit	114	56.6	2.2	5	116	116	12.3	0.63	1	35
Descent to water	108	6.6	1.5	1	45	117	2.8	0.19	1	12
Total time	**30**	**148.2**	**7.3**	**94**	**276**	**12**	**68.83**	**6.12**	**36**	**101**

Table 7.3.1. The mean duration of each stage of the nesting process in minutes with standard errors, sample sizes and ranges for *C.mydas* and *C.caretta* on Alagadi beach.

A correlation matrix was constructed, to examine the relationships of the duration of each of the nesting behaviours with the other behaviours. No significant relationships were recorded for either species (Pearsons Product Moment $p>0.05$).

7.3.5. Chelonia mydas

Female size, in any of its three measures, was not related to any behavioural stage of nesting. These results are given in table 7.3.2. In the comparison made between nesting behaviour and reproductive output only two significant relationship were recorded, see table 7.3.3. Both hatching success and hatchling emergence success were significantly related to the time spent digging the egg chamber.

The longer the time spent digging the egg chamber the lower the resultant success of the nest. This relationship is illustrated in figure 7.3.3. None of the variables examined were related to the total time spent on the beach by a female.

A further comparison was made of the times spent during each stage of nesting when nests subsequently hatched or failed to hatch. Using a t-test, the only difference found between the two groups was in the time spent digging the body pit. This was significantly shorter (14.3 ± 2.4 minutes, n=6) in females whose nest failed to hatch (t=-2.48, p=0.031, n=30) compared to those which hatched (21.58 ± 1.7 minutes, n=24).

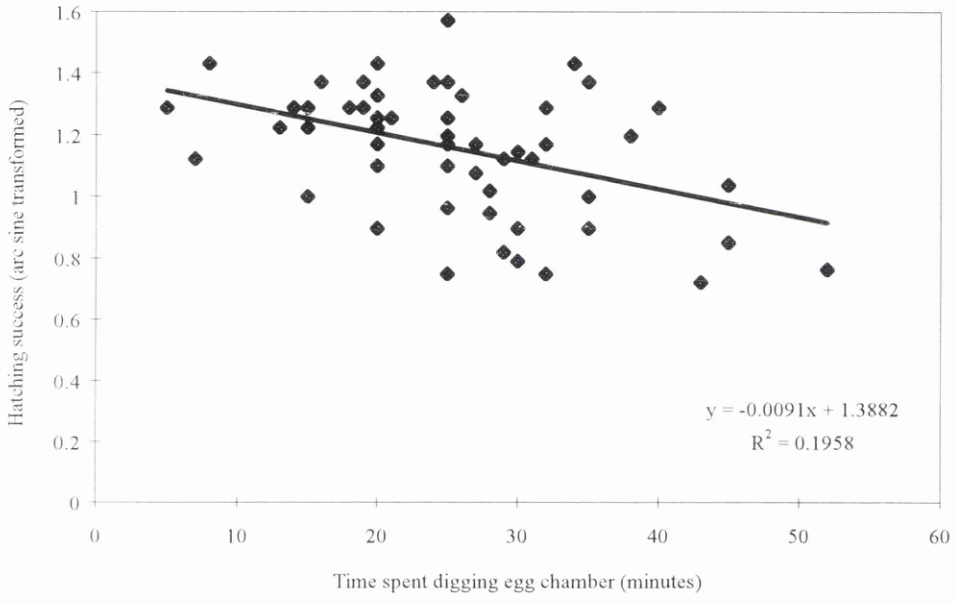


Figure 7.3.3. The relationship between the time spent digging the egg chamber (minutes) and the resultant hatching success of a nest, presented here as the *arcsine* of hatching success (%), for *C.mydas*.

Behaviour	*Female PCA$_{adult\ size}$*			*Curved carapace length (cm)*			*Curved carapace width (cm)*		
	Statistic	*Probability*	*N*	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Ascent	F= 0.69	0.415	26	F=1.23	0.352	26	F= 0.43	0.63	26
Dig body pit	F= 2.06	0.159	44	F=1.68	0.202	44	F=1.92	0.174	44
Dig egg chamber	F= 1.13	0.292	47	F=0.39	0.533	47	F=2.26	0.137	47
Lay clutch	F= 1.18	0.280	49	F=2.13	0.148	49	F=0.38	0.538	49
Cover egg chamber	F= 0.99	0.323	49	F=0.61	0.438	49	F=1.29	0.260	49
Cover body pit	F=0.46	0.497	50	F=0.12	0.731	50	F=0.94	0.335	50
Descent	F= 1.74	0.199	49	F=1.02	0.131	49	F=2.04	0.09	49
Total time	F= 0.44	0.511	26	F=0.32	0.618	26	F=1.31	0.113	26
Hatching success	r= 0.01	0.942	48	F=0.01	0.920	48	F=0.01	0.919	48
Emergence success	F= 0.25	0.621	48	F=0.13	0.718	48	F=0.32	0.575	48
Clutch size	F= 33.01	< 0.0005*	48	F=32.14	<0.0005*	48	F=26.11	<0.0005*	48

Table 7.3.2. The relationship between the duration of the stages of nesting and the size of *C.mydas* females nesting at Alagadi.

	Clutch size			*Hatching success (%)*			*Hatchling emergence success (%)*		
Behaviour	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Ascent	F= 0.16	0.689	22	r= 0.104	>0.05	22	r= 0.24	>0.05	22
Dig body pit	F= 1.89	0.232	43	r= 0.263	>0.05	33	r= 0.33	>0.05	33
Dig egg chamber	F= 2.83	0.189	76	F= 13.52	0.001*	61	F= 6.34	0.009*	60
Lay clutch	F= 1.72	0.39	82	F= 0.36	0.553	75	F= 0.57	0.512	75
Cover egg chamber	F= 1.72	0.231	92	r= 0.062	>0.05	80	r= 0.171	>0.05	80
Cover body pit	F= 1.39	0.371	92	r= 0.058	>0.05	83	r= 0.11	>0.05	83
Descent	F= 0.71	0.41	84	r= -0.352	>0.05	71	r= 0.443	>0.05	71
Total time	F= 0.59	0.451	23	r= 0.109	>0.05	23	r= 0.039	>0.05	23
Hatching success	r= -0.023	>0.0.5	48	-	-	-	-	-	-
Emergence success	F= 1.17	>0.05	47	-	-	-	-	-	-
Clutch size	-	-	-	F= 0.06	0.802	48	F= 1.17	0.285	48

Table 7.3.3. The relationship between the duration of the stages of nesting and reproductive output of *C.mydas* females nesting at Alagadi.

7.3.6. Caretta caretta

A summary of the statistical comparisons made with their results and significance level are given in table 7.3.4 and 7.3.5. for *C.caretta*. Sample sizes for the times spent in ascending the beach, digging the body pit and the total time spent on the beach were too small for a statistical comparison to be made with female size, clutch size, hatching success and hatchling emergence success.

Female size and hatching success were not significantly related to the duration of any of the stages of nesting behaviour in *C.caretta*. A significant positive relationship was however recorded between the time spent laying and the clutch size and is illustrated in figure 7.3.4.

In a comparison between the time spent at each nesting stage and whether a nest hatched or failed to hatch, only one variable was significantly different. This was the time spent making the descent from the nest site to the water and was longer (3.19 ± 0.28 minutes, n=69) in females whose nests failed to hatch than those which successfully hatched (2.25 ± 0.21 minutes, n=48, t=-2.69, p=0.008, n=117).

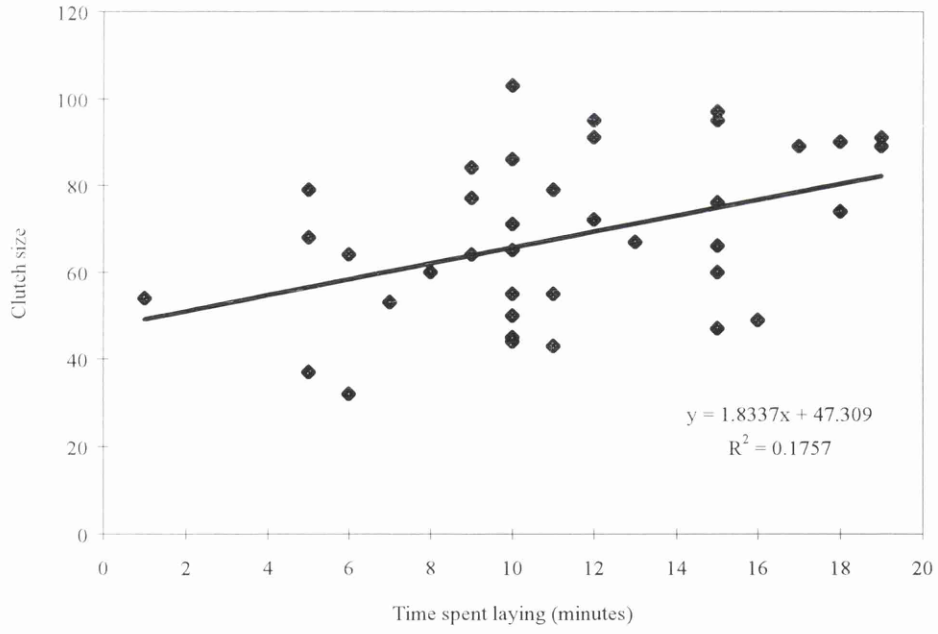


Figure 7.3.4. The relationship between the time spent laying the eggs (minutes) and the clutch size of *C.caretta* females.

Behaviour	*Female PCA$_{adult\ size}$*			*Curved carapace length (cm)*			*Curved carapace width (cm)*		
	Statistic	*Probability*	*N*	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Dig egg chamber	F= 0.82	0.370	36	F= 0.97	0.331	36	F= 0.6	0.444	36
Lay clutch	F= 0.62	0.435	47	F= 1.38	0.244	47	F= 0.09	0.760	47
Cover egg chamber	F= 0.74	0.392	53	F= 1.51	0.222	53	F= 0.19	0.664	53
Cover body pit	F= 1.26	0.264	55	F= 1.27	0.262	55	F=2.49	0.118	55
Descent	F= 0.58	0.449	62	F= 1.27	0.263	62	F=0.12	0.728	62
Hatching success	F= 0.28	0.602	40	F= 0.7	0.408	40	F=0.01	0.91	40
Emergence success	F= 0.19	0.663	40	F=0.32	0.574	40	F=0.32	0.575	40
Clutch size	F= 14.53	<0.0005*	41	F= 11.3	0.002	41	F=26.11	<0.005*	41

Table 7.3.4. The relationship between the duration of the stages of nesting and size of *C.caretta* females nesting at Alagadi.

Behaviour	*Clutch size*			*Hatching success (%)*			*Hatchling emergence success (%)*		
	Statistic	*Probability*	*N*	*Statistic*	*Probability*	*N*	*Statistic*	*Probability*	*N*
Dig egg chamber	F= 1.93	0.180	21	F= 0.5	0.489	22	F=1.76	0.201	21
Lay clutch	F= 12.24	0.001*	43	F= 1.22	0.276	45	F= 1.21	0.277	44
Cover egg chamber	F= 1.04	0.312	47	F= 0.69	0.411	46	F= 0.38	0.541	46
Cover body pit	F= 1.67	0.203	47	F= 0.37	0.547	47	F= 0.2	0.656	46
Descent	F= 0.49	0.489	47	F= 2.81	0.102	48	F= 2.78	0.051	45
Hatching success	F= 0.02	0.894	41	-	-	-	-	-	-
Emergence success	F= 0.11	0.740	40	-	-	-	-	-	-
Clutch size	-	-	-	F=0.02	0.894	41	F= 0.08	0.779	41

Table 7.3.5. The relationship between the duration of the stages of nesting and reproductive output of *C.caretta* females nesting at Alagadi.

7.3.7. Effects of tagging females on nesting behaviour

A one way ANOVA was used to assess the effect of tagging females on behaviour. The time spent covering the egg chamber, covering the body pit and descending the beach were compared when a female had been; i) tagged on one flipper only, ii) tagged on both flippers and iii) not tagged at the time of observation; having been tagged on a previous occasion.

For *C.mydas*, no significant difference was recorded between any of the three groups for the time spent covering the egg chamber ($F= 0.23$, $p=0.792$, $n=107$), covering the body pit ($F=1.46$, $p=0.236$, $n=110$) or descent of the beach ($F=1.3$, $p=0.201$, $n=108$)

Similar results were recorded for *C.caretta* with no differences observed in the duration of the three behavioural stages ($F=0.98$, $p=0.378$, $n=96$; $F=0.64$, $p=0.529$, $n=101$; $F=2.69$, $p=0.073$, $n=102$) respectively. These results are displayed in figures 7.3.5. and 7.3.6. for *C.mydas* and *C.caretta* respectively.

In nearly all instances the times recorded in each of the nesting stages were longer in females that were not subjected to tagging. However the durations were not shorter if a female was tagged twice as opposed to only once.

The three groups were also examined in relation to hatching success of the nest. For *C.mydas* nests, no significant differences were found ($F=0.51$, $p=0.602$, $n=88$). Similar results were recorded with respect to *C.caretta* ($F=0.6$, $p=0.521$, $n=49$)

Most females were tagged after laying their first clutch and were only tagged subsequently if they had lost a tag, or were only tagged once during their initial lay. Thus, to examine the behavioural changes that occur as the number of clutches a female lays increases, the duration of behaviours during clutches 1-4 were examined. No significant difference was recorded for either *C.mydas* ($F=1.03$, $p=0.384$, $n=107$; $F=1.62$, $p=0.189$, $n=110$; $F=1.53$, $p=0.152$, $n=108$) or *C.caretta* ($F=1.46$, $p=0.230$, $n=96$; $F=0.33$, $p=0.806$, $n=101$; $F=1.15$, $p=0.334$, $n=102$) for the three 'post laying behaviours listed above.

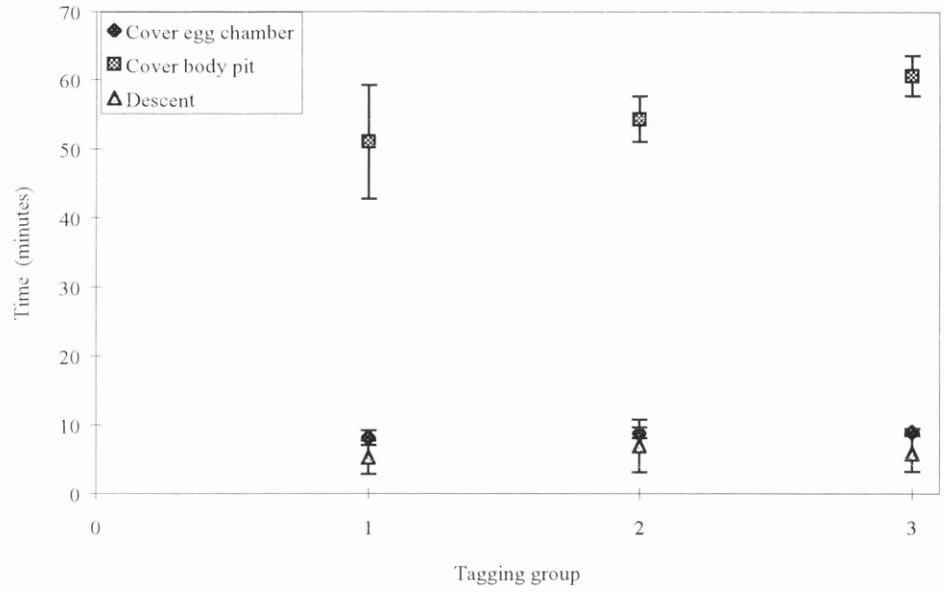


Figure 7.3.5. The mean time (with standard error bars) spent in each of the three behavioural stages if tagged once (1), tagged twice (2), or if not tagged (3) for *C.mydas* females.

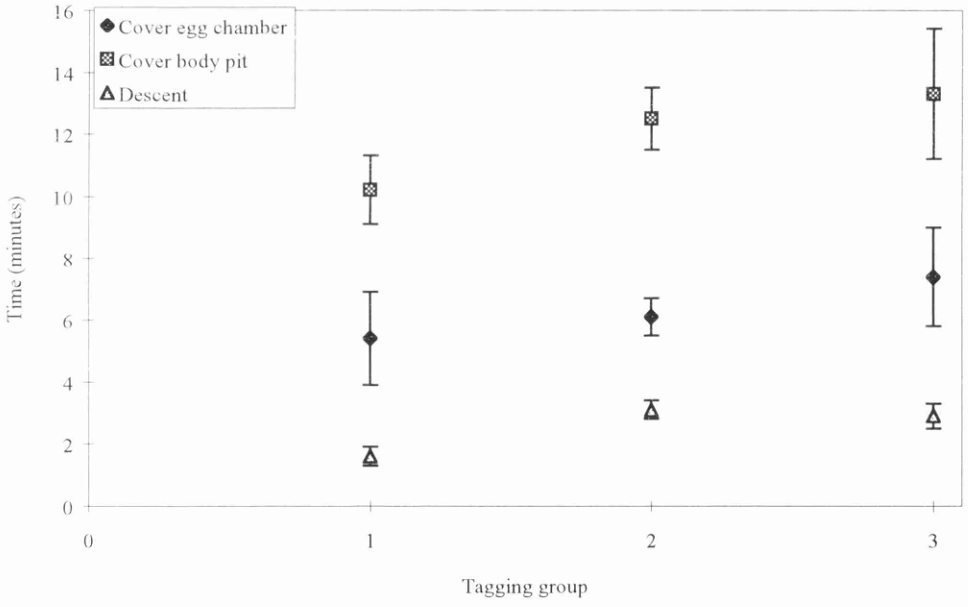


Figure 7.3.6. The mean time (with standard error bars) spent in each of the three behavioural stages if tagged once (1), tagged twice (2), or if not tagged (3) for *C.caretta* females.

7.4. Discussion

To minimise costs associated with nesting, females must complete nesting before dawn when temperature and brightness increase. Whether a change in temperature or light intensity acts as a signal to begin or end nesting is not known. Thus it might be expected that selection would favour females coming onto the beach earlier in the night to ensure that they complete the process before dawn. If however they are only investigating different sites in preparation to nest on another night this is not as important. *C.mydas* spends, on average, more than twice as long completing nesting than *C.caretta*, however peak emergence of females going on to nest successfully is not earlier in *C.mydas*, as might have been expected. However, although a smaller proportion of nesting *C.mydas* females emerge after 01:00 there are no great differences found in the timing of activities between the two species with respect to any of the three activities.

On land, the different gaits of *C.mydas* and *C.caretta* may be explained by looking more closely at those used by all species of marine turtle. The three largest species; *D.coriacea, C.mydas* and *N.depressa* move both flippers simultaneously. It is possible that this movement generates the required force to overcome the inertia of their greater mass. In contrast, *E.imbricata, C.caretta, L.kempi* and *L.olivacea* alternate the sequence of flipper movements, a process sufficient to move their smaller forms.

The size of body pit varies between species. *C.mydas* makes a deep body pit, but *N.depressa* does not (Ehrhart 1982). *E.imbricata, C.caretta,* and *L.olivacea* make shallow pits whilst *L.kempi* makes none at all (Ehrhart 1982). In addition, variation also exists between populations of the same species, for example, *D.coriacea* nesting in Costa Rica prepare a substantial body pit but those nesting in Surinam do not. Larger females can dig their egg chambers to a greater depth (chapter 5) therefore one would expect that larger species of marine turtle would have less need to dig deep body pits to lower themselves prior to digging their egg chamber. This trend does not appear to exist. Examination of the distance nests are laid from the waters edge, by different species on the same beach, may explain some variation.

A reduced success may be expected for those that lay excessively shallow nests being at risk from damage, desiccation and/or of detection by predators. However variations found

both between and within beaches with respect to sand quality and topography may account for these species and population differences.

Ehrhart (1982) notes that in *E.imbricata, C.caretta, L.kempi* and *L.olivacea* the hind flippers are spread beside the nest during laying and their medial edges curl as eggs are extruded. In *D.coriacea, C.mydas* and *N.depressa* the hind flippers generally cover the nest cavity and remain at rest as eggs are extruded. Although this behaviour has been observed in Cyprus, it does not appear to be the case in all instances.

No information on previous studies of the duration of the stages of behaviour for *C.mydas* females nesting has been found in the literature, hence no comparison can be made. However, Dodd (1988), in his global synopsis, reported that *C.caretta* females spend approximately 6 - 10 minutes digging their body pit, 10 - 15 minutes digging the egg chamber, 7 - 25 minutes laying, 10 - 15 minutes covering the egg chamber and 26 - 45 minutes covering the body pit. The mean duration of behaviours recorded for *C.caretta* all fall within the ranges presented by Dodd (1988), with the exception of the time spent covering the body pit which is shorter in Cyprus, both in means and ranges.

More recently, Hays and Speakman (1991) recorded the mean duration of nesting behaviours of *C.caretta* nesting on the Greek island of Cephalonia as: ascent- 7.75 minutes, dig body pit - 10.96 minutes, dig egg chamber - 24.72 minutes, lay - 25.2 minutes, cover egg chamber and body pit 20.12 minutes. *C.caretta* nesting in Cyprus appear to invest less time in covering their clutch than those in Greece. Possibly this is related to the larger clutch laid by this species in Greece. Certainly, population variations are to be expected as environmental conditions vary spatially.

As indicated in chapter 4, in the Mediterranean *C.mydas,* on average, is a larger species than *C.caretta* and has a larger clutch size. The former species may thus invest more time and energy in ensuring the clutch is incubated under the correct conditions, which may explain, in part, why *C.mydas* spends, on average, more than twice as long on the beach digging, laying and covering her clutch. The greatest difference between the two species was in the time spent covering over the body pit, which in *C.mydas* was, on average, almost five times longer than in *C.caretta*. *C.mydas* digs a deeper nest and so needs to use more sand in covering it over. However, this process may also mask the scent and position

of the nest, making it harder for predators to detect. Indeed, as will be shown in chapter 8, fewer *C.mydas* nests are predated in comparison to *C.caretta* nests.

Spending a greater time on the beach completing the nesting process however, will subject the female herself to a greater risk from predators. At the Göksu Delta in Turkey, golden jackals (*Canis aureus*) have been reported to kill adult *C.caretta* females whilst on the beach (Peters & Verhoeven 1992). Although *C.mydas* females also nest at the Göksu, none have been reported to have suffered the same fate. Whether the fact that fewer *C.mydas* are found in this location is the sole reason for these observations remains to be seen but it is possible that the smaller adult size of *C.caretta* females makes them more vulnerable to predators. In Cyprus, jackals are not found and, although foxes may come onto the beach, there has been no recorded incidence of adult mortality due to predation.

The olive ridley (*Lepidochelys olivacea*), nesting in Costa Rica, may attempt to overcome the threat of predation by its nesting behaviour. Some females have synchronised nesting, or 'arribadas', whilst others are solitary nesters. The nests of the latter have been shown to suffer a greater loss as a result of mammalian predators than those laid in 'arribadas' (Eckrich & Owens 1995; Kalb & Owens 1994). Thus predator satiation may be occurring. This may similarly be the case where adults are predated.

Analyses of nesting data collected from observations of *C.mydas* females showed that when less time was spent digging the egg chamber, hatching success and hatchling emergence success were high. The simplest explanation for this relationship is that when the sand quality is poor, due to its moisture content or particle size, constructing the egg chamber is more difficult and thus takes longer. If such problems are experienced with building, this may mean that the site is sub-optimal for embryonic development leading to a poor hatching success in the nest. Any delay in oviposition may further influence hatching success. In the chicken, egg retention results in excess calcification and the production of a tougher shell which is more difficult to pip (S.E. Solomon, pers. comm.).

The fact that there was no relationship between the time spent digging the egg chamber by *C.mydas* females and whether the nest hatched or failed, regardless of the proportion of eggs which succeeded, is difficult to explain with respect to the above theory. However if the time spent digging the egg chamber reaches a certain threshold, females may abandon the site which may explain why this relationship is not found in this instance. Perhaps it is

only that larger, possibly more experienced nesters, have greater success in their nest selection and viability of clutches. Certainly when observing these nesting processes the impression is gained that *C.caretta* females are not as selective in choosing their nest sites; thus the latter will frequently nest at the waters edge.

However, if one examines the number of false crawls that were recorded in each species (chapter 3), the proportion of the overall *C.mydas* activities that resulted in successful nestings was 35% whereas in *C.caretta* was 45%. This so called adult emergence success is often given in the literature as an indication of the suitability of a nesting beach. High emergence success may indicate that females are having few problems with the nest sites (Coley & Smart 1992). However in such a comparison as this, between species at the same site, such results may be an indication, as stated previously, that *C.caretta* females are less selective in choosing their nesting sites. This may in part explain the slightly higher hatching rate of nests in *C.mydas* over the years in comparison to *C.caretta* (chapter 4).

There was no relationship between the total time spent on the beach and either the clutch size, hatching success or hatchling emergence success, indicating that *C.mydas* females do not invest more time and energy in digging or covering their nest if they are laying a larger clutch. No relationship was recorded between the duration of laying and the size of the clutch, in contradiction to the results for *C.mydas* in Costa Rica presented by Campbell (1994).

Unlike *C.mydas*, no relationship was found between the time spent digging the egg chamber by *C.caretta* females and hatching and hatchling emergence success. Two relationships were however recorded with respect to reproductive output of *C.caretta* females. One was the positive relationship between the time spent laying and the size of the clutch. This was not found in *C.mydas* and may be a result of an anatomical or physiological constraint. This relationship has also been recorded for *C.caretta* females nesting on the Greek island of Cephalonia (Hays & Speakman 1991). There was also a significant difference recorded in this present study between the duration of the descent of *C.caretta* females and the resultant success or failure of their nests to hatch. Descent times were significantly longer in nests which completely failed to hatch. This is interesting in the light of the study by Johnson *et al* (1996) which found that turtles

observed by groups took a "less direct route to the water after completion of nesting"; although no reduced success of the nest was recorded in these cases.
In some instances, females were unable to find a suitable nest site on emerging from the water and moved up the beach. They may have nested too far away from the waters edge in drier sand which caused the nests to fail, alternatively these longer descent times may not have been a result of a greater distance but the speed of the female. For example, less experienced females may take longer to reach the water. Perhaps those that take longer in their descent are doing so as a result of disturbance, trying to move away from observers. If they were also observed by onlookers during other stages of the nesting process this may have affected the quality of the nest in some way and subsequently the success.

From the results herein, there does not appear to be any significant effect on the behaviour of nesting females as a result of tagging. This is strengthened by the fact that the duration of behaviours of females tagged twice and those not tagged, were more similar than those tagged once and those not tagged. Although a non-significant reduction in the duration of these behaviours is seen in tagged versus untagged females, a similar trend is also seen with an increase in the number of clutches a female lays. Although not a significant relationship, the more clutches a female laid, the longer she spent during the post laying stages of nesting. Similarly, no effect of tagging females was recorded with respect to the resultant success of their nests. However one cannot at this stage preclude any long term effects. Females may be more likely to nest elsewhere in their subsequent clutch or season as a result of being tagged.

As ecotourism becomes more popular more 'turtle tours' are taken to observe marine turtles nesting. It is very important to keep a track of how such tours may affect the behaviour of females and the success of their nests, although in many places without such a tourist interest the species would not be protected. Similarly whilst carrying out research it is important to remember that the researcher may be subjecting an animal to stress that affects it detrimentally. It was for this reason that during this study adult females were not weighed when ashore nor were their eggs removed to be counted or measured.

In comparison to *C.caretta*, *C.mydas* females invest much more time and energy in creating a suitable environment for clutch development. *C.caretta* females and their nests may be at a greater risk of predation. Although they invest less time in their nest by spending less time on the beach they may reduce the risk of predation to themselves or

their nest. Although predators may not attack females themselves they may observe nesting and thus be attracted to the site of the nest making location of the eggs easier. There may thus be a trade off between spending time covering the nest and camouflaging it from predators and being observed doing so. The shallower nest and smaller adult size of *C.caretta* may make an easier target for predators.

The strategies seen in these two species may not exist in areas where they are not found nesting side by side. For example if *C.caretta* nests are more prone to predation than *C.mydas* nests, *C.mydas* females may adopt a different strategy, in light of a possible reduced threat to their nests. Similarly where predation is very high and adult predators are also found on land, strategies may differ.

A key factor in these analyses is that, as far as can be determined, the female has no knowledge of the subsequent success of her nest and so modifying behaviour to maximise the success of a nest would be impossible. Such behaviours must be inherited, rather than learnt. It may however be possible that as a female gains experience in nesting she learns of areas of beach which are easier to dig and returns to these places each year. This may explain why the nests of larger females have a higher hatching success. Alternatively, as females increase in size they lay larger clutches and it may be these that are more successful rather than the site or effect of nesting behaviours.

Two explanations are proposed for the mating behaviours observed during this study, one being the theory of mate guarding the other a sneaking male present. Since these observations occurred during the first 2-4 weeks of the nesting season it might be hypothesised that these were prior to the deposition of the first clutch by the females concerned. Two of these observations may have been a result of the pair being incidentally washed ashore as a result of stormy weather. However, on the other two occasions the females went on to attempt nesting or complete the process. This suggests that the females in question were making a concerted effort to reach the nesting beach. Mating at this late stage is unlikely to have any fitness benefit to either the male or female. Any sperm transferred to the female are likely to be flushed out at oviposition. There is also a possibility that this male had previously mated with this female and was guarding his mate until she had reached the safety of the beach, reducing the chance of further copulations. However if there is little chance of another male fertilising her clutch at this late stage then why guard the female?

It is possible that these observations involved the same 'rogue' male, who, unable to compete with other males for matings prior to the onset of the season, was using an unorthodox 'sneaking' strategy (Krebs & Davies 1987). Thus he was seeking copulations with females as they approached the beach to nest. Whilst he would be expected to have a lesser chance of fertilising clutches than males that had mated with females previously, he may still have stood some chance of fertilising, at least some of the eggs, of future clutches. Nesting green turtles in Northern Cyprus range in curved carapace length from 79 -106 cm (chapter 4). Thus, although no information is available on the size of male *C.mydas* turtles in this region, it is possible that this male is smaller (CCL 85 cm) than average, which might support the theory that he was 'sneaking' copulations.

Additional observations of mating *C.mydas* turtles have been made off the coast of Northern Cyprus, although none have been made of *C.caretta* turtles mating. Possibly the mating grounds of the latter species are not within this vicinity or their mating behaviour is such that they are observed less frequently.

The success of nests and eggs and possible reasons for their failure are discussed further in chapters 8 and 9.

Chapter 8 - The Fate of *Chelonia mydas* and *Caretta caretta* nests at Alagadi.

8.1. Introduction

Of the numerous threats to marine turtle nests, many are due to man's activities. These include beach development, generally as a result of tourism, sand extraction, egg harvesting and pollution. These factors were introduced in chapter 1. There are also natural threats, such as predation, inundation or infestation of nests. Some may result in total nest loss, whilst others may only affect a few eggs in the clutch, allowing the remainder to hatch successfully. These 'natural' threats are examined in this chapter.

World-wide, a great many species have been recorded as predating marine turtle eggs and hatchlings on land. These range from crabs and snakes, to crows and vultures, racoons, coatis, skunks and feral cats (Fowler 1979; Hopkins *et al.* 1978; Mora & Robinson 1984; Seabrook 1989; Snow 1982). In the Mediterranean, the main predators of marine turtle nests are the red fox (*Vulpes vulpes),* golden jackals (*Canis aureus*), feral dogs and ghost crabs (*Ocypode cursor*) (Brown & Macdonald 1995; Godley *et al.* 1996; Margaritoulis *et al.* 1996a; Peters *et al.* 1994a). At Akyatan, in Turkey 75% of *C.mydas* nests were predated by jackals or foxes (Brown & Macdonald 1995). Where such high levels of nest loss occur, nest protection is necessary.

Most anti-predation programmes involve either moving nests into safe areas or removing the predator (Stancyk *et al.* 1980). The highest hatching success rates however come from nests left *in situ* and it is, therefore desirable to leave nests in their original sites where possible (Eckert & Eckert 1990; Limpus *et al.* 1979). Nests can be protected *in situ* by placing screens or cages above the nest so that the predator cannot reach the eggs. This method has been shown to have dramatically reduced nest loss in Greece (Margaritoulis *et al.* 1996a). Some studies have examined the use of taste aversion to decrease predation levels with mixed success (Hopkins & Murphy 1982; Nicolaus *et al.* 1982).

Nests may also be vulnerable as a result of their position on the beach. Laid either close to or below the high water mark they are at risk from inundation or erosion as a result of storms. In St. Croix (US Virgin Islands), Eckert (1987) reported than between 45 and 60% of leatherback nests were lost each year as a result of beach erosion. Where total flooding occurs from wash-over of sea water or heavy rainfall with poor drainage, embryos at all stages of development have been shown to suffocate (Kraemer & Bell 1980; McGehee 1990). It is not known to what extent embryos can survive a lesser degree of over-wash. It is possible that

females select elevated sites to nest avoiding areas of poor drainage and thus decreasing the possibility of suffocation.

The particle size of the sand, to an extent, dictates its moisture holding capacity. It is also important in permitting gaseous exchange and building of the egg chamber. However, different species and even populations of the same species, nest on a variety of shore types (Stancyk & Ross 1978). As well as the selection between beaches these factors may influence the females choice to nest on certain areas of beaches. Female size may restrict the maximum depth to which the nest can be dug, limiting the possible range of moisture and particle conditions available to nesting females.

Mortimer (1990) analysed sand particle size on *C.mydas* nesting beaches and showed that most of these beaches around the world had sand particle diameters of between 0.2-1.0 mm. However, previous studies have shown that not only was there a wide range in sand types used by nesting females, but there was no relationship between particle size and nesting success (Hughes 1974; Stancyk & Ross 1978; Chen & Cheng 1995). Mortimer (1982a) did however find that there was an optimum range of grain sizes for hatching success, but that females did not appear to select for this range. Mortimer (1990) also recorded a decrease in the hatching success of nests as the porosity of the sand increased.

The moisture content of the sand has also been shown to affect the hatching success of nests. McGehee (1990) reported that hatching success was highest in *C.caretta* nests with moisture levels of 25% and lowest at 100%. Incubation periods also increased with an increase in moisture. In addition, moisture content has been shown to affect hatchling size with eggs incubated at higher temperatures and lower moisture content, producing smaller hatchlings and cooler moister sand producing larger hatchlings (Gutzke *et al.* 1987).

The tropical freshwater turtle (*Chelodina rugosa)* of Australia, has been shown to nest underwater (Kennett *et al.* 1993). Embryonic development was found to remain arrested until the flood waters receded. These recent findings may be of interest when considering the above results.

Few studies have been conducted on the effect of insect infestation of marine turtle eggs. Lopes (1982) identified *Eumacronychia sternalis* (Diptera, Sarcophagidae) living on eggs of *C.mydas* nesting on the Pacific coast of Mexico. It was recorded as having infested nearly

90% of nests transplanted to hatcheries as well as some *in situ* nests. Their data indicated that infestation reduced hatching success by at least 30%.

Other studies have documented larvae of the Dipteran family Phoridae, specifically *Megaselia scalaris,* in nests of *C.mydas* (Fowler 1979) and *E.imbricata* (Bjorndal *et al.* 1985) in Costa Rica. Several studies have shown that larvae of phorids also infest eggs of fresh water turtles (Acuña-Mesen & Hanson 1990; Vogt 1981) and a review of these is given by Iverson and Perry (1994). In the Mediterranean, only coleopteran larvae have been reported infesting eggs of *C.caretta* in Turkey (Baran & Türkozan 1996). No other published data exist on insect larval infestation of marine turtle nests in the Mediterranean.

It is important to monitor all variables that might have an effect on hatching success in an attempt to reduce their influence on endangered populations. The large clutch deposited by marine turtles may not compensate for a persistent loss of eggs. In this chapter, the fate of nests, and eggs within those nests are examined and the relationship with sand quality is explored.

8.2. Methodology

8.2.1. The fate of nests

The fate of nests was recorded according to the protocol described in chapter 2. Nests were identified as either;

1) hatched with no sign of predation,
2) hatched and predated - evidence of hatching is recorded either from tracks or nest remains together with predator disturbance,
3) totally predated with no sign of hatching,
4) predated and hatched - prior to hatching the nest was predated in part and the remains salvaged reburried and hatched.

In 1995, due to the large number of *C.caretta* nests which failed to hatch, an attempt was made to investigate the causes of nest failure. As a result of the high level of human usage it was not possible to externally mark nests. It was therefore necessary to measure out the position of the nests and dig within that area in an attempt to find the eggs.

8.2.2. The fate of eggs

From a selected number of hatched nests, unhatched eggs were incised and their contents categorised as follows; 'undeveloped', 'hatched', 'dead in shell' and 'non-viable' as previously defined in chapter 2. Predated and transplanted nests are not included in the data.

8.2.3. Sand quality analysis.

Sand samples were collected from all hatched nests in 1995. In addition, sand was also taken from the walls of egg chambers of a selection of nesting females. In the event of a nest failing to hatch, these samples were used for comparison with sand from nests which hatched. All samples were immediately placed in air tight bags.

a) Moisture content

100g of sand was left to dry in the sun over 2-3 days until the weight remained constant. This weight loss represents the amount of water lost from the sample, i.e. the moisture content of the sand in the nest.

b) Porosity

Larger samples of sand were dried to examine porosity. 250 ml (V1) of dry sand were placed into a measuring cylinder into which was added 250 ml (V2) of water, giving a total volume of 500 ml (V3). The sample was left for a few minutes to allow all the water to infiltrate the sand and a recording of the new water level was taken (V4). V3-V4 represents the amount of water taken up by the sand (V5). By the calculation V5/V3 a measure of porosity on a scale of 0-1 was generated.

c) Particle Size

An aliquot of sand from each sample nest was also taken back to Glasgow for particle analysis by sieving. 50g of sand was dried overnight and then shaken mechanically through sieves of mesh diameter: 1 mm, 500 μm, 355 μm, 250 μm, 180 μm, 125 μm. The amount of sand trapped by each sieve and the pan (< 125 μm) was then weighed.

Statistical analysis.

A computer programme was used to obtain values for mean particle size, sorting, and skewness of the sand samples. Using a one way ANOVA, a comparison of the sand quality in nests of the two species which hatched was possible. In addition *C.caretta* nests which failed to hatch were also compared. Regression analysis was used to examine sand quality in relation to the hatching success, hatchling emergence success and incubation period of a nest.

8.2.3. Insect infestation of nests

Work was carried out at the main nesting site at Alagadi, supplemented by data collected at other beaches along the coast. During excavation of nests after hatching, insect larvae were sometimes discovered in the remains. The number of infested eggs was recorded and the larvae taken to the laboratory to rear. Samples were kept in glass jars covered with muslin at room temperature and checked at 48 hour intervals. When adult insects were observed they were left for at least 24 hours to allow cuticles to harden before they were removed and fixed in alcohol. Samples were then returned to the U.K. for identification. In some instances, other invertebrates were recorded in the nest and either identified immediately or preserved for later study.

8.3. Results

8.3.1. The fate of nests

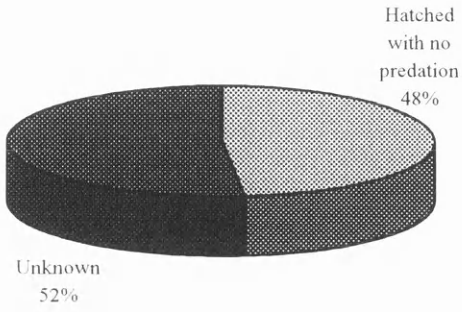
Figure 8.3.1. illustrates the fate of all nests laid in each of the three years of this study for the two species. In 1995, 90% of *C.mydas* nests were recorded as having hatched. In this year, only three nests were affected by predation. It is interesting to note that in 1993 and 1994, when no predation was observed, fewer *C.mydas* nests successfully hatched in comparison to the level recorded in 1995.

In general *C.caretta* nests were subject to higher predation levels. Thus in 1993 and 1994 when no predation activity was recorded for *C.mydas*, the levels for *C.caretta* were 2% and 7% respectively. In 1995 this level rose to 33%, however not all of these nests were totally destroyed by predation. In 10% of nests, predation occurred post-hatching, whilst in 14% of predated nests, remains were salvaged and hatching of the latter successfully occurred.

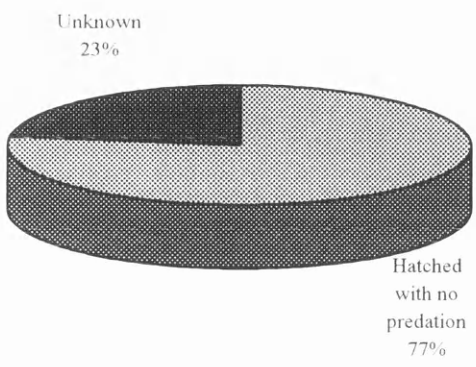
The temporal distribution of predation is illustrated in figure 8.3.2. A peak is seen toward the end of July. This was the point at which hatching began in 1995.

In 1995 an attempt was made to locate all unhatched nests. For reasons of manpower involvement it was only possible to conduct this survey during the 1995 season. No *C.mydas* nests were recorded to have experienced any over-wash. However 19 *C.caretta* nests had been inundated. This represents 20% of the total nests laid and a large proportion of the fate of 'unknown' nests in 1995.

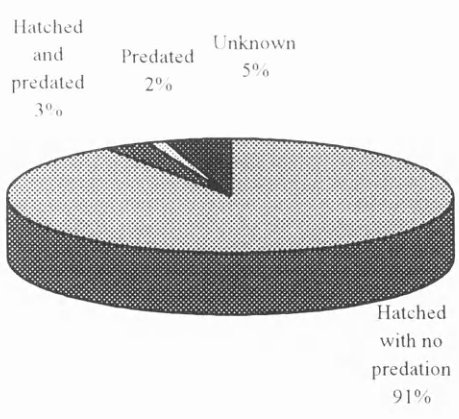
In 1995, 3 *C.mydas* and 10 *C.caretta* nests were relocated, having been laid too close to the waters edge. All three of the *C.mydas* nests hatched with a mean success of 65% (± 22.5%, n=3). Of the 10 *C.caretta* nests relocated, 2 failed to hatch and three were part predated but hatched at a later date. The remaining five relocated *C.caretta* nests successfully hatched with a success rate of 53% (± 6.8%, n=5).



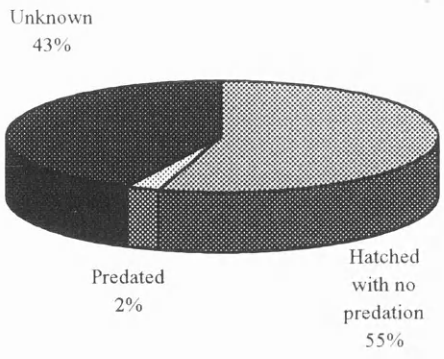
a) *C.mydas* 1993.



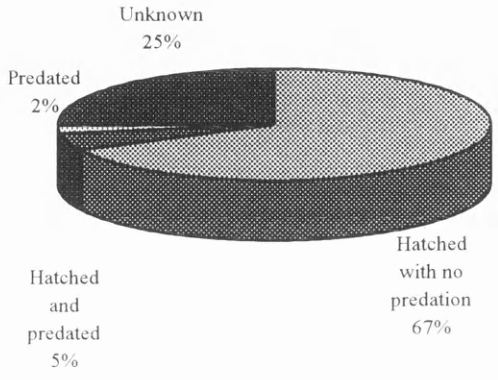
b) *C.mydas* 1994.



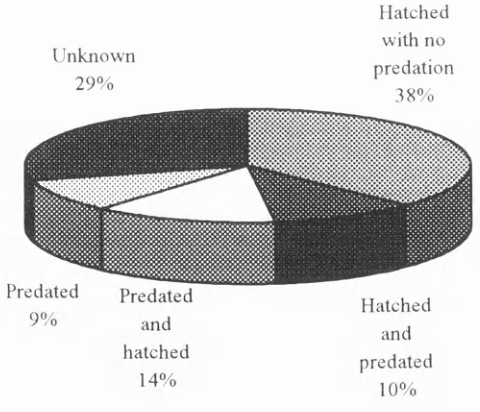
c) *C.mydas* 1995.



d) *C.caretta* 1993.



e) *C.caretta* 1994.



f) *C.caretta* 1995.

Figure 8.3.1. a-f). The fate of *C.mydas* and *C.caretta* nests laid at Alagadi, 1993-1995.

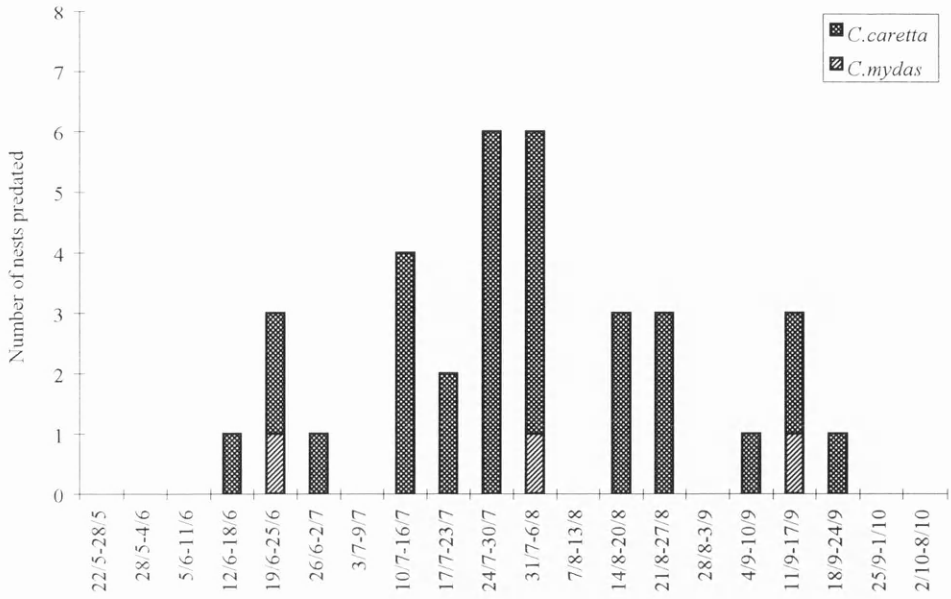
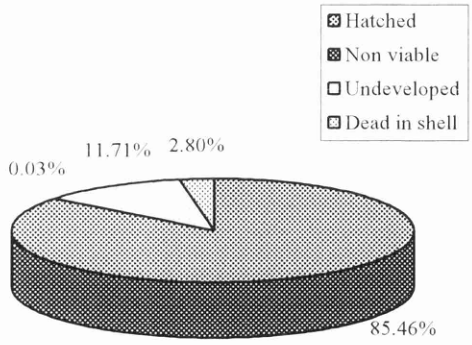


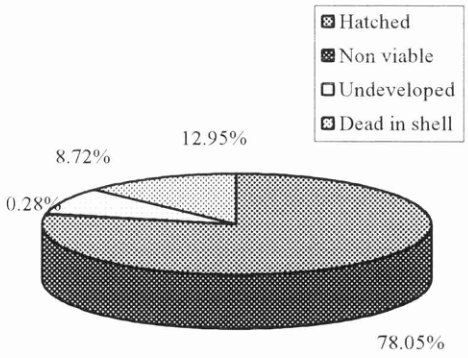
Figure 8.3.2. The temporal distribution of predation of *C.mydas* and *C.caretta* nests on Alagadi Beach in 1995.

8.3.2. The fate of eggs

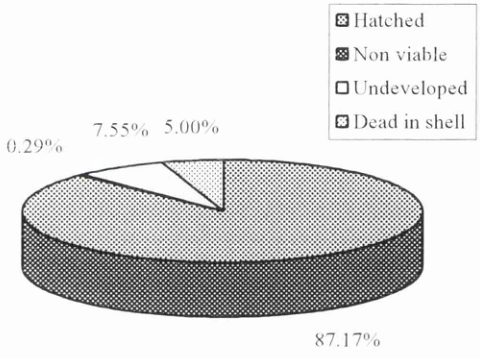
Figure 8.3.3. illustrates the fate of eggs within the hatched nests of both species in each of the three years of this study. Hatching success was greater within the nests of *C.mydas* although an anticipated variation did occur from year to year. The hatching success of *C.caretta* nests was more variable over the three years, ranging between 69-85%. This was due to an increase in the proportion of 'undeveloped' eggs and 'dead-in-shells'. Although the number of 'undeveloped' eggs in hatched nests was fairly similar between the two species, the higher percentage of 'dead-in-shells' recorded in *C.caretta* nests accounts for most of the species variation recorded. A slightly higher proportion of *C.caretta* eggs were 'non-viable', nevertheless, in both species these eggs never represented more than 1% of the clutch.



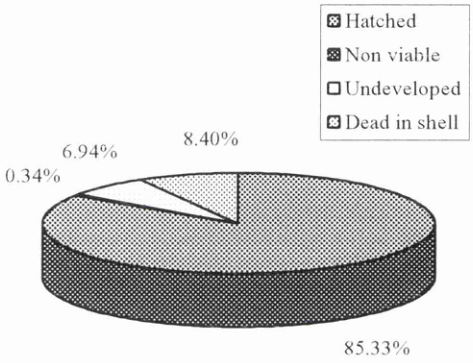
a) *C.mydas* 1993.



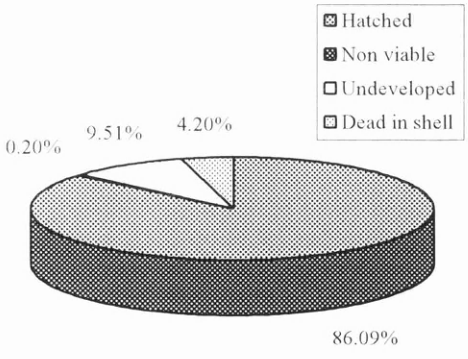
b) *C.caretta* 1993.



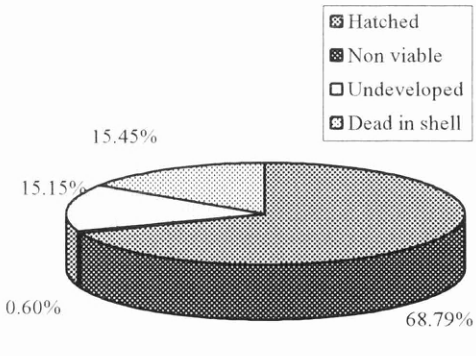
c) *C.mydas* 1994



d) *C.caretta* 1994



e) *C.mydas* 1995



f) *C.caretta* 1995

Figure 8.3.3. a-f) The fate of eggs within nests of *C.mydas* and *C.caretta* laid at Alagadi, 1993-1995.

8.3.3. Sand quality analysis

The high success rate of *C.mydas* nests in 1995 (60 out of 64), invalidated the use of statistical analyses to compare sand samples of unhatched nests with those that hatched. However, the mean particle size of sand collected from *C.mydas* hatched nests was 0.262 mm in diameter (± 0.025, n=22) with a skewness of -0.074. According to the Wentworth grade for classification of soil particles, the mean particle size found in *C.mydas* nests was of 'medium sand' and was classified as moderately well sorted. A skewness of -0.074 indicates that the distribution of the particles was symmetrical (Holme & McIntyre 1984).

The mean particle size of sand collected from *C.caretta* hatched nests was 0.245 mm in diameter (± 0.028, n=24) with a skewness of -0.0792. Particles of this diameter are classified as 'fine sand' and similarly the samples were moderately well sorted and symmetrically distributed (Holme & McIntyre 1984). The mean particle size of sand collected from unhatched *C.caretta* nests was 0.248 mm in diameter (± 0.041, n=11), with a skewness of -0.1148. This sand type is also classified as 'fine sand' which is well sorted although a skewness of -0.1148 is classified as being skewed toward more coarser sand particles. The proportions of the sand sample in each of the particle size brackets are illustrated in figure 8.3.4.

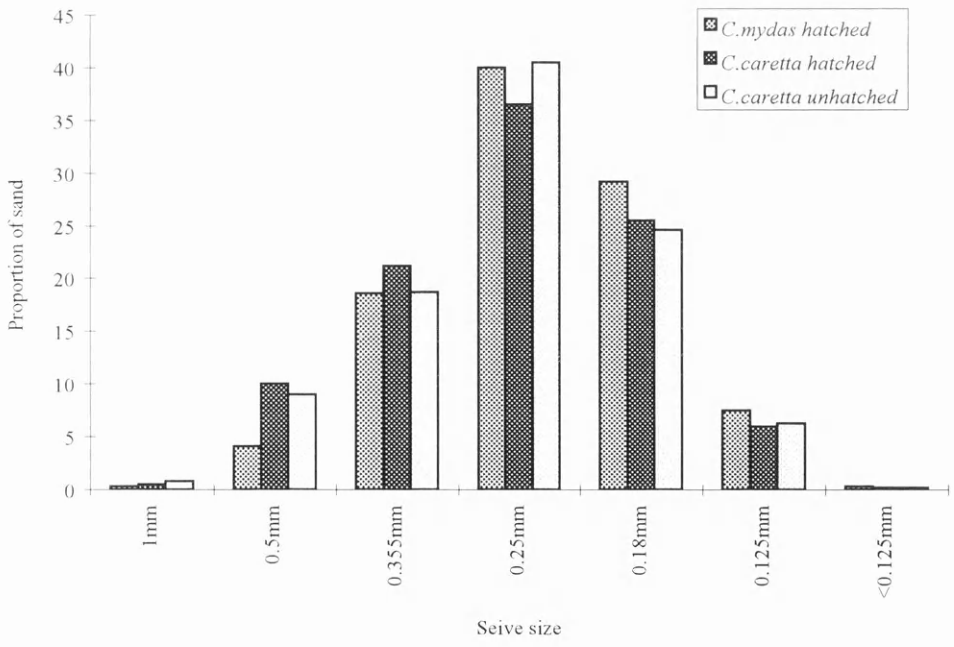


Figure 8.3.4. Particle size of sand from within hatched nests of *C.mydas* and *C.caretta* and unhatched nests of *C.caretta*.

A comparison was also made between the weight of sand recorded in each of the seven sieves and the three categories (*C.mydas* hatched nest, *C.caretta* hatched nest and *C.caretta* unhatched nest). These results are illustrated in table 8.3.1. No significant differences were found. Similarly, no significant differences were recorded in either the moisture content (F=1.28, p=0.290, n=43) or porosity (F=0.34, p=0.716, n=43) levels in either *C.mydas* hatched, *C.caretta* hatched or *C.caretta* unhatched nests.

	Statistical results of one way ANOVA		
1 mm	F=1.63	p=0.205	n=56
0.5 mm	F=2.52	p=0.09	n=56
0.355 mm	F=0.59	p=0.558	n=56
0.25 mm	F=1.0	p=0.373	n=56
0.18 mm	F=0.98	p=0.383	n=56
0.125 mm	F=1.15	p=0.325	n=56
pan	F=0.85	p=0.432	n=56
Mean particle size	F=0.81	p=0.452	n=56

Table 8.3.1. Statistical results of a one way ANOVA comparing the weight of sand in each sieve in the three categories of sample (*C.mydas* hatched, *C.caretta* hatched, *C.caretta* unhatched).

Using regression analysis, the relationship between the mean particle size, moisture content and porosity of sand in a nest were compared to the hatching success, hatchling emergence success and incubation period of that nest. In both *C.mydas* and *C.caretta* nests, no significant relationships were recorded between the mean particle size or porosity of the sand and the hatching success, hatchling emergence success or incubation period. These results are summarised in table 8.3.2. and 8.3.3.

	Mean Particle size			Porosity			Moisture content		
Hatching success (%)	F=0.14	p=0.71	n=19	F=1.16	p=0.30	n=14	F=6.22	p=0.03	n=12
Hatchling emergence success (%)	F=0.03	p=0.87	n=19	F=1.38	p=0.26	n=15	F=2.65	p=0.04	n=15
Incubation period (days)	F=0.9	p=0.36	n=17	F=0.62	p=0.45	n=13	F=0.23	p=0.64	n=11

Table 8.3.2. Results of regression analysis to compare sand quality with the success and incubation period of a *C.mydas* nest.

	Mean particle size			Porosity			Moisture content		
Hatching success (%)	F=0.01	p=0.91	n=13	F=0.48	p=0.51	n=11	F=0.1	p=0.76	n=12
Hatchling emergence success (%)	F=0.05	p=0.83	n=13	F=0.24	p=0.64	n=11	F=0.02	p=0.88	n=12
Incubation period (days)	F=0.22	p=0.64	n=21	F=0.93	p=0.35	n=18	F=9.06	p=0.008	n=19

Table 8.3.3. Results of regression analysis to compare sand quality with the success and incubation period of a *C.caretta* nest.

In *C.mydas* nests, however, the moisture content of the sand was related to both the hatching success and hatchling emergence success of the nest. In both cases, these relationships were positive. The relationship between moisture content and hatching success is illustrated in figure 8.3.5. No significant relationship was recorded between the incubation period of *C.mydas* nests and their moisture content.

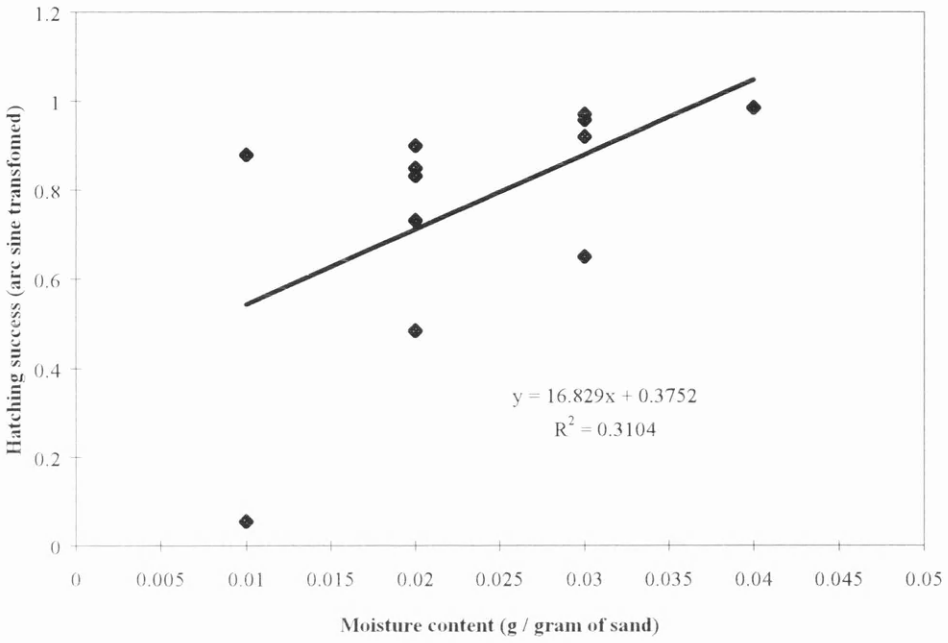


Figure 8.3.5. The relationship between the moisture content recorded in *C.mydas* nests and the resultant hatching success of the nest.

A significant and positive relationship was also recorded between the moisture content in sand of *C.caretta* nests and the incubation period of the nest, and is illustrated in figure 8.3.6. There was no significant relationship however between the moisture content of *C.caretta* nests and their hatching or hatchling emergence success.

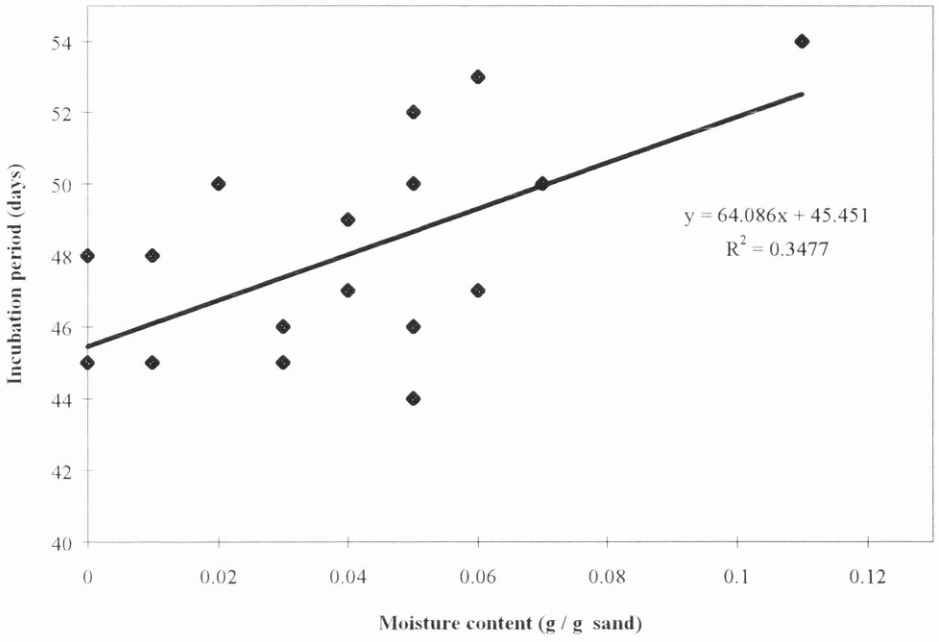


Figure 8.3.6. The relationship between the moisture content recorded in *C.caretta* nests and the resultant incubation period of the nest.

8.3.4. Insect infestation of nests

In 1995, eleven (23%) of the hatched *C.caretta* nests at Alagadi were found to be infested with insect larvae. In *C.mydas* nests, insect larvae were recorded in 5 (9%) of the nests. In these clutches, infestation had occurred in 4.6% (± 1.45, n=5) of *C.mydas* eggs and 10.6% (± 4.81, n=11) of *C.caretta* eggs. Infested nests were also found at other beaches, although their effects were not quantified.

Adult insects were successfully reared from seven nests. These samples were identified as containing four species of flies from two different families (Diptera; Sarcophagidae and Phoridae) and one species of wasp, *Brachymeria podagarica* (Hymenoptera; Chalicidae). The sarcophagids are *Sarcophaga (Parasarcophaga) crassipalpis, Sarcotachina subcylindrica* and a species of *Wohlfahrtia,* possibly *indigens*. The phorid was the well known polyphagous species *Megaselia scalaris* which is world-wide in its distribution.

S.(P.) crassipalpis and *S. subcylindrica* were found in the nests of both species of turtles. *M. scalaris* was reared from a *C.caretta* and the *Wohlfahrtia* sp. from a *C.mydas* nest. The wasp is known to be a widespread parasitoid of muscoid flies, including the Sarcophagidae (Beaver 1986) and, in this instance, was found in association with *S.(P.) crassipalpis* in a *C.mydas* nest.

During earlier work in 1994, two other sarcophagids were recorded, both of which were reared from *C.caretta* nests. These were a species of *Sarcophaga (Parasarcophaga)*, either *argyrocephala* or *hirtipes*, and an as yet unidentified member of the tribe Amobiini, subfamily Miltogramminae. Other observations include one beetle larvae (Coleoptera; Scarabaeidae), which was not identifiable to species level, found in a *C.mydas* nest. In addition, one of the nests with fly larvae also contained an antlion larva (Neuroptera; Myrmeleonidae) and in several other nests enchytraeid worms (Annelida) were recorded in eggs of *C.caretta*.

8.4. Discussion

Hatching success varies annually, as factors such as weather, nest site location and predation each take their toll, either in combination or singly. In 1993, the hatching rate for both species was approximately 50%. In that year, erratic climatic conditions including high winds may have resulted in nests being flooded. The same conditions would have undoubtedly made the detection of hatchling tracks more difficult.

The mere presence of man is generally sufficient to ward off predators. Nevertheless, in 1995 at least one fox, together with cubs, was observed on the beach during the night surveys. It is thought that this fox was responsible for the majority of predation that occurred in this year. Caging the nests subsequent to these observations undoubtedly improved hatchability but did not alter the fact that from the nest to the water the hatchlings were at risk from predation.

On one occasion, a *C.mydas* female was observed to expose her newly deposited clutch as she attempted to cover her nesting site. This vulnerable nest was subsequently predated, the only *C.mydas* nest to be predated prior to hatching in the three years of this study. The proximity of a clutch to the surface of the sand must also have a role to play in the ease with which predators can reach their prey; thus the shallower *C.caretta* clutches incurred a higher level of predation. In addition, predators may become satiated by feeding on accessible *C.caretta* nests thus ignoring *C.mydas* nests. To this end it is interesting to note that on beaches around the island where the population of *C.caretta* is low compared to *C.mydas*, nests of the latter are frequently predated (Broderick & Godley 1995).

Although, in Cyprus, rainfall during the summer months is very rare and is not considered a threat to marine turtle nests, wash-over, due to wind storms, does occur, particularly during September and October. The shallower nests of *C.caretta* are particularly vulnerable, located closer to the waters edge. Indeed in a study on the Greek island of Cephalonia, Hays and Speakman (1993) found that the further the *C.caretta* nest was from the sea the greater the hatching success of the nest. In 1995, at Alagadi, 20% of *C.caretta* nests experienced some degree of inundation. Although the proportion of nests which incurred flooding was not examined in the previous years of this study it is likely to have been a cause for the failure of some *C.caretta* nests.

Whitmore and Dutton (1985) found that *D.coriacea* nests in Surinam had a higher embryonic mortality and infertility than *C.mydas* nests. Nests of *D.coriacea* are also shallower that those of *C.mydas*. Eggs at the top and bottom of the clutch are spatially distinct and the environment in which they are developing is comparably different with specific reference to temperature and moisture (Kaska *et al.* 1997). These variables might adversely influence the developmental process and thus explain the higher embryonic mortality recorded in *C.caretta* nests. Relocation proved successful and although the success reached did not match the *in situ* values, it is important to remember that these nests were designated doomed and so any success must be looked upon as an increase in the recruitment of hatchlings to the population.

The particle size of sand in *C.mydas* nests and those of *C.caretta*, both hatched and unhatched, were very similar in their qualities. Although sand from *C.mydas* nests was classified as medium and that of *C.caretta* nests as fine, the division between the two was marginal. This slight variation may be a result of the deeper nests of *C.mydas*, nevertheless, over the beach there appears to be little variation in particle size. The mean particle size recorded in *C.mydas* nests however is within the range of 0.2-1 mm quoted by Mortimer (1990) for *C.mydas* nesting beaches around the world. The only variation seen between the sand quality of *C.caretta* hatched and unhatched nests was in the skewness of the particles. In unhatched nests sand particles had a greater proportion of coarser grains of sand and consequently might be expected to display different physical properties.

Monitoring the degree of insect infestation with any accuracy requires excavation of nests during the incubation period. Such intrusion is to be avoided and so, as with many other aspects of this study, a compromise situation is necessary. The ease with which burrowing insects will reach a nest will be governed by many factors, not the least of which is nest depth. Deep within the confines of their pit, the eggs of *C.mydas* are protected to some extent by location which also serves to mask their scent.

At any time from oviposition to hatching insects may infest the nest. At the time of laying, eggs are exposed and cloacal secretions may act as an attractant. Infrequently, flies have been observed landing on eggs at this time. However if the nest was infested at this point the larvae discovered at hatching must have been of a subsequent generation, as the life cycle of all the species recorded in this study is shorter that the 42 - 60 days incubation period for

development (Busvine 1980; Disney 1994). It is possible that these insects laid their eggs or larvae into the sand and these burrowed to the nest.

Members of the family Sarcophagidae, known colloquially in English as flesh-flies, are strongly attracted to both freshly dead and decomposing animal tissue. They are viviparous, the female producing active first instar larvae. It is probable that the flies deposit their larvae on the sand which then burrow to the food source. This has been observed and experimentally demonstrated with another species that is predatory on earthworms (Lopes 1982). Several members of the Phoridae are able to burrow as adults through the soil, up to a depth of one metre, in order to lay their eggs on buried corpses (Disney 1994). It is believed that turtle nests are exposed to potential infestation by insects at any time during the development of the embryo, despite being buried immediately after laying.

Lopes (1982) indicates that insect infestation is much higher in transplanted *C. mydas* nests. This may be due to exposure or damage to some of the eggs during this process, concentrating the nests in a small area, or a combination of these factors. To reduce the latter problem, a number of separate hatchery sites, relocated each season, might be considered as a preventative measure. The observations of the parasitoid wasp, *Brachymeria podagarica*, indicate that, in Cyprus at least, it may exert some natural biological control over the sarcophagid fly infestations. Whether or not this insect could or should be encouraged in artificial hatcheries is debatable, but may be worth investigation.

These infestations may be primary factors in the reduction of the success of a nest or only affect moribund eggs or embryos. Their attraction to the nest in the first instance is highly likely to be the result of the smell of decay, whether they will go on to attack viable eggs after utilising their initial food source is not known. In a study on *Graptemys pseudogeographica*, a species of freshwater turtle, Vogt (1981) recorded infestation in eggs containing live hatchlings and suggested that most infestation occurred at pipping. Whilst it seems possible that infestation of nests poses a direct threat to incubating clutches, these insects may, in effect, remove decaying material from the nest so decreasing the risk of further infestation.

Although levels of infestation are given for those nests which were hatched and excavated, it is not known to what extent unhatched nests may have been infested. In addition, a nest may have been infested at an earlier point during incubation and the larvae migrated away from the

nest to pupate, thus going unrecorded. The levels of infestation presented in this thesis are therefore a minimum.

Once laid in the sand, the clutch is left to develop unaided by maternal influences. In the event that all the environmental criteria within the nest are suitable, the developmental process should proceed unhindered, but the embryo is developing not only within a sandy egg chamber but within a calcitic chamber manufactured within the maternal oviduct. The properties of the eggshell have a crucial role to play in embryonic survival and are considered in the following chapter.

Chapter 9 - Eggshell Quality.

9.1. Introduction

Thus far, this thesis has concentrated on the role of the external environment on the reproductive effort. Parameters such as temperature, sand condition and nest site have all been shown to impinge on the latter, to a greater or lesser extent. But the reproductive female exerts another controlling factor on the process of hatchability, prior to abandoning the clutch within the nest, and that is the quality of her eggshell.

The reptilian eggshell, like its avian counterpart, serves a number of distinct functions. It protects the embryo from shock, provides nutrients, and controls O_2 and CO_2 exchange. The eggshell is also crucial in protecting the embryo from invading micro-organisms. The latter can act upon the eggshell causing crystal dissolution, thus increasing the porosity of the shell which in turn decreases its strength (Ferguson 1981).

Scanning Electron Microscopy (SEM) studies have shown that the marine turtle eggshell consists of calcium carbonate in its aragonite modification, interspersed occasionally with calcite (Baird & Solomon 1979). The calcium carbonate layer is attached to a fibrous soft shell membrane. An excess of calcite may interfere with the development of the embryo, by reducing the porous nature of the shell.

The aragonite nature of the eggshell gives it a great degree of flexibility, a property which is essential to minimise trauma as the egg is dropped into the nest. Flexibility is also essential to permit water uptake. In birds the transfer of 'plumping fluid' is effected within the shell gland pouch. This fluid causes the albumen to swell and stretches the soft shell membranes (Solomon 1991). The reptilian eggshell attains its final size through the addition of water from the sand, i.e. outwith the oviduct. Water is free to move across the entire shell surface. Likewise in the absence of discrete gas exchange pores (Solomon & Watt 1985), O_2 and CO_2 are not spatially restricted in their movement. It has been suggested that cracks and fissures in the shell may act as pore like structures for transport of gases and water (Packard & Hirsch 1986).

During incubation, the turtle embryo withdraws calcium from the eggshell, thereby reducing its thickness (Bustard *et al.* 1969; Carthy 1994). Solomon and Tippett (1987) observed, in the eggshells of farm reared *C.mydas* turtles, an excess of the calcite form of $CaCO_3$ and subsequently observed the same phenomenon in wild populations. They

noted a higher incidence of fungal hyphae associated with the calcitic type shell and drew attention to the fact that these fungal hyphae were compromising the hatchling in two distinct ways. Firstly, by forming a web beneath the calcite layer, they were interfering with gas exchange and secondly, their ability to withdraw calcium form the shell was reducing the efficiency of the latter as a calcium source for the embryo.

It is acknowledged, from the extensive studies on avian eggshells with respect to bird strain, housing and diet, that the eggshell can be detrimentally influenced by external factors (Solomon 1991). The changes in avian shell structure range from variation in colour, through to gross deformities, which render the shell useless both as an embryonic chamber and as a consumer product. The process of avian eggshell mineralisation is controlled by an organic matrix which exists as a framework within the shell complex. Although the inorganic and organic fractions of the eggshell precipitate simultaneously, there is a variation in the morphology of both fractions from the inner surface of the eggshell outwards. Bird age also appears to influence the structure and distribution of the organic layer and it has been hypothesised that some of the deterioration in this fraction is the result of liver dysfunction (Fraser 1996).

Chan and Solomon (1989) demonstrated structural variations in the eggshells of the leatherback turtle (*D. coriacea*) nesting in Malaysia. These turtles were dissuaded from nesting because of the presence of tourists and so returned to the sea without depositing their clutch.

This chapter does not set out to be definitive in its findings, but to alert the reader to the fact that, at every stage of the life cycle the turtle embryo is constrained by factors outwith its control, but which have a crucial role to play in the development process. This chapter considers the morphology of the inorganic component of the eggshell and gives a brief consideration of the influence of the organic fraction of the eggshell on the process of mineralisation.

9.2. Methodology

9.2.1. Scanning Electron Microscopy (SEM).

Thirty shells from 'undeveloped', 'hatched', 'dead in shell' and 'non-viable' eggs of each species were washed and allowed to dry. Samples were then bagged for transport to the laboratory. The shells were then saturated with water and pinned out on cork board to dry. 2-5 flat pieces from each shell were cut and fixed to aluminium stubs using silver paint. Samples and stubs were coated with gold/palladium in an EMSCOPE sputter coater. The specimens were examined using a Phillips 501 B scanning electron microscope at 15 kV. Shells from the above categories were examined to determine any inter-species variation. In addition analysis of 'undeveloped' eggshells permitted an evaluation of structural changes during the laying season unimpeded by the development process.

9.2.2. X-ray microanalysis

The elemental composition of a selection of samples was assessed using the EDAX PV 9100 X-ray Microanalyser system with the SEM set at 15 kV. Samples were examined, coated and uncoated with gold/palladium, and were attached with double sided tape to aluminium stubs. Coating can interfere with the collection of signals from the specimen and so distort the findings.

9.2.3. SDS-Page

Recent technological advances and the availability of expertise, provided an ideal opportunity to carry out a pilot study on the potential role and variation in composition of the organic fraction of the mineralised layer of the turtle eggshell.

The loose outer crystalline layer of 'undeveloped' eggs was used for these analyses, having maintained their original structure. This analysis was carried out by Joel Gautron (INRA France), using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). This technique has recently been reviewed by Fraser (1996).

9.2.4. Amino Acids analyses.

Pooled samples of 'undeveloped' eggshells from 5-10 hatched nests of both species, laid in weeks 2, 4 and 6 (corresponding to beginning, middle and end of lay) of the 1994 nesting season were treated as follows.

The loose outer crystalline layer of the eggshell was removed and 35-41 mg of this material was dissolved in 11 µls of 2N HCL/1mg. The sample was then shaken until dissolved (approx. 30 minutes) and spun down to remove any insoluble matter. The sample was loaded onto a Model 420A Derivatizer for PTC amino acid analysis. Following hydrolysis, which frees the amino acids, derivatization was accomplished by coupling the amino acid group with PITC (Phenylisothiocyanate) in the presence of DIEA (N, N-diisopropylethylamine). The derivative was extracted, and subsequently analysed by HPLC (high pressure liquid chromatography). This analysis was carried out by Dr. Maggie Cusack in the department of Geology, University of Glasgow.

In this analysis, the results obtained are given as Pmols/mg. However, due to unbalanced loading of the system, the results are presented for each amino acid as a percentage of the overall sample.

9.3. Results

9.3.1. Scanning Electron Microscopy.

9.3.1.1. General structure

The marine turtle eggshell consists of an inner organic membrane (figure 9.3.1.) upon which an outer inorganic layer of calcium carbonate exists in the form of aragonite (figure 9.3.2.). These aragonite crystals originate from organic nucleation sites and radiate out in a needle like form. Further crystal growth gives rise to an outer randomly arranged layer (figure 9.3.3.).

During embryonic development, calcium is withdrawn from the eggshell leaving it fragmented (figure 9.3.4.).Calcite blocks do not appear to undergo any breakdown and are frequently observed on the soft shell membranes at hatching (figure 9.3.5.).

At the beginning and end of oviposition the female frequently deposits abnormally shaped eggs. The latter are usually small and without their yolk mass. Nevertheless they do appear to go through the process of mineralisation. Figure 9.3.6. illustrates the distorted appearance of the inorganic aspect of such a shell.

9.3.1.2. Seasonal variation in the structure of the eggshell.

a) C.mydas

Clutch 1

An attempt has been made by the female to deposit eggs of the normal configuration (figure 9.3.7.) with associated cuticular material (figure 9.3.8.). Nevertheless, within this pattern of normality, calcite blocks are frequently observed (figure 9.3.9.). Within the latter, individual crystals are growing in a rhombohedral form (figure 9.3.10.). Fungal hyphae are also present sending their radiating strands into the inorganic fraction (figure 9.3.11.).

Clutch 2.

The images obtained from the second clutch in the season are consistent with those observed in the first, i.e. aragonite with occasional calcite inclusions (figures 9.3.12. - 9.3.14.).

Clutch 3.

Mid lay, the eggshell appears less organised. The earlier defined nature of the aragonite spicules is now superseded by a more molten and fragmented outer covering (figure 9.3.15.).

Clutch 4

At the end of lay some eggs within the clutch still retain the appearance of 'normality' (figure 9.3.16.), whilst others have acquired a heavy deposit of amorphous material which serves to obscure the aragonite crystals (figure 9.3.17.).

b) C.caretta

Clutch 1

Both the aragonite (figure 9.3.18.) and calcite (figure 9.3.19.) forms of $Caco_3$ were observed within the eggshell of *C.caretta*. In contrast to the shells of *C.mydas* however, the outermost layer of the eggshell of *C.caretta* has a nodular arrangement of aragonite, i.e. is more organised (figure 9.3.20.). It is interesting to note that the lozenge shaped crystal masses observed on the surface of eggshells of the leatherback by Chan and Solomon (1989) are also present in the eggshells of *C.caretta*. These structural forms were not observed in the eggshells of *C.mydas* (figure 9.3.21.).

Clutch 2.

The second clutch of the season provides images consistent with those observed earlier (figure 9.3.22.).

9.3.2. X-ray microanalysis

X-ray microanalysis demonstrated the presence of a variety of elements within the eggshells. In coated samples there are readily discernible peaks for Aluminium (Al) (most probably from the stub on which the sample is based), Mercury (Hg), Cadmium (Cd) and Calcium (Ca). The Ca peak however, is much higher in *C.caretta* (figure 9.3.23.) than *C.mydas* (figure 9.3.24.). Hg and Cd were still discernible, although to a lesser degree in uncoated specimens (figure 9.3.25.)

9.3.3. SDS-Page

The intra and extra mineral protein fractions of eggshells from both species are illustrated in figure 9.3.26. With respect to the intra mineral fraction, both display bands at 66, 26-27, 22, 14 and 6-5 kDa. Within the extra mineral fraction, the 6-5 kDa band is a common feature, although the 66 kDa band appears to be specific to *C.caretta*. Band 14 has recently been identified by Nys (pers.comm.) as Lysozyme.

9.3.4. Amino Acids analyses

Figures 9.3.27. and 9.3.28. illustrate both the species, and seasonal variation in amino acid levels in the outer crystalline layer of the turtle eggshell. Sixteen amino acids were recorded in the eggshells of both species. In the eggshell of *C. mydas,* although levels fluctuate in the three samples, no overall trend can be seen. A dramatic decrease is however observed in the levels of serine as the season progresses. In the eggshells of *C.caretta*, many more of the amino acids recorded decrease after week 2, suggesting that in these cases stores of these amino acids are becoming depleted. Intra-species differences were observed, thus the levels of serine recorded are markedly different, with much higher levels recorded in week 2 for *C.mydas* but higher levels recorded in weeks 4 and 6 for *C.caretta*. Large variations in valine and glutamic acid are also noted.

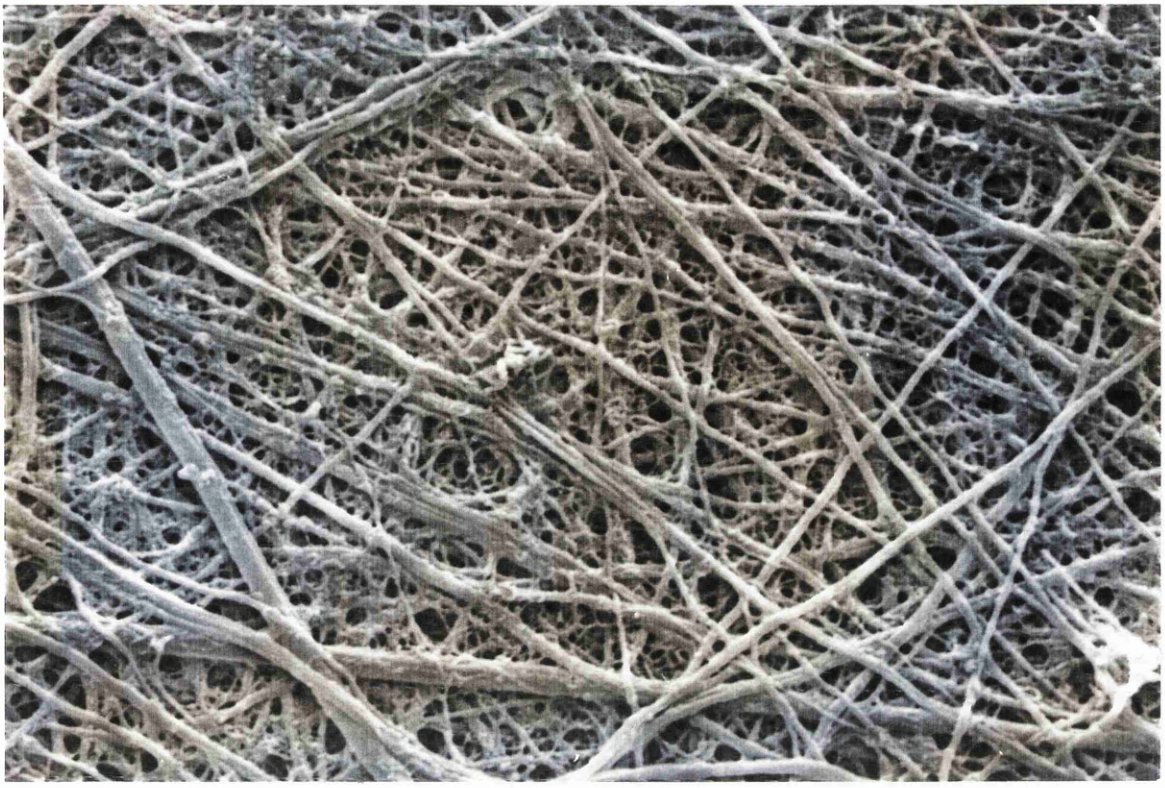


Figure 9.3.1. The inner organic membrane of the eggshell of *C.mydas*, x 1250.

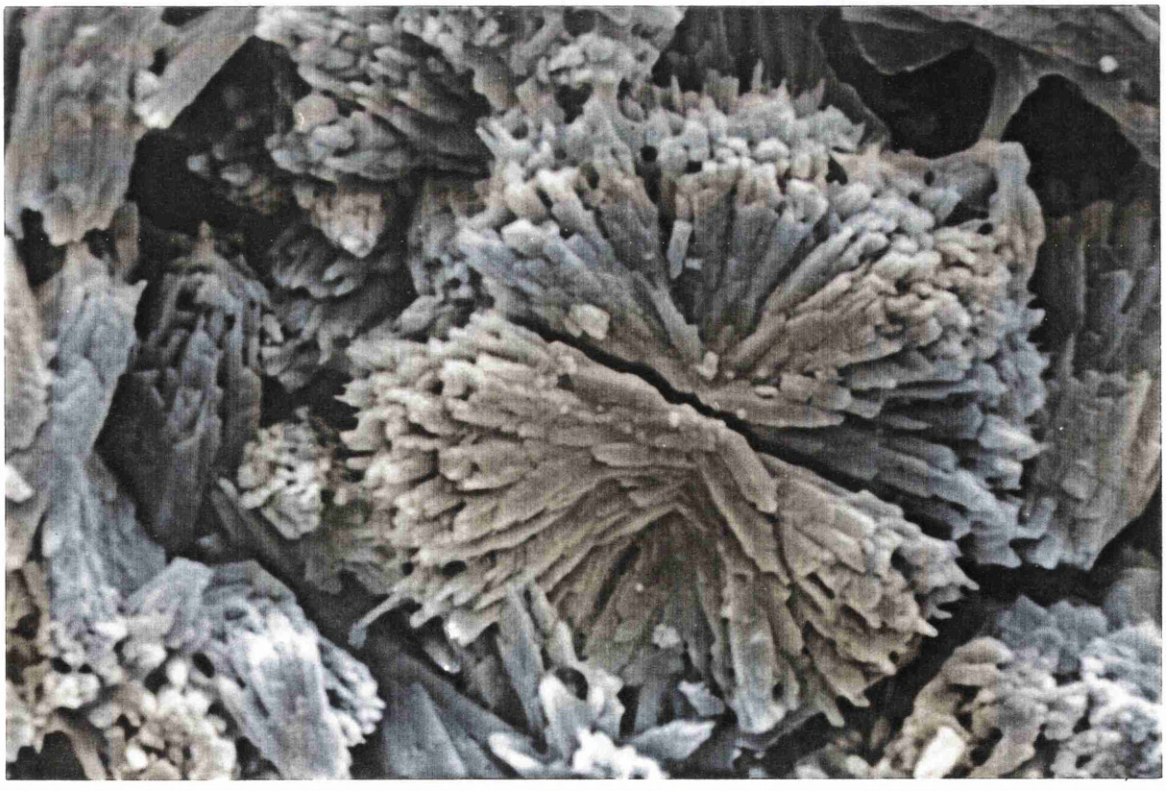


Figure 9.3.2. *C.mydas* undeveloped eggshell, demonstrating radial growth of aragonite crystals, x 1250.

Figure 9.3.3. Typical aragonite structure of the eggshell of a *C.caretta* undeveloped egg, x 640.

Figure 9.3.4. *C.caretta*, dead in shell, showing the erratic crystal pattern due to calcium reabsorption, x 1250.

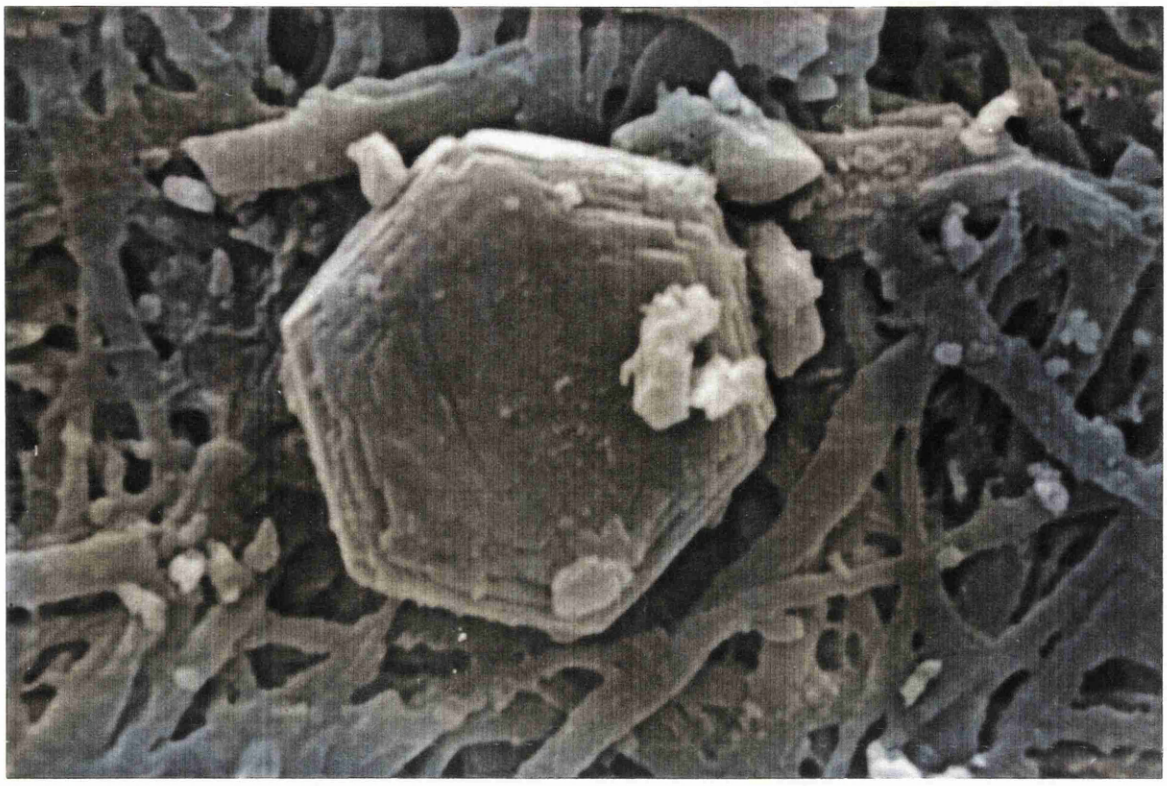


Figure 9.3.5. *C.caretta* hatched eggshell. A block of calcite lying on the inner membrane, x 2500.

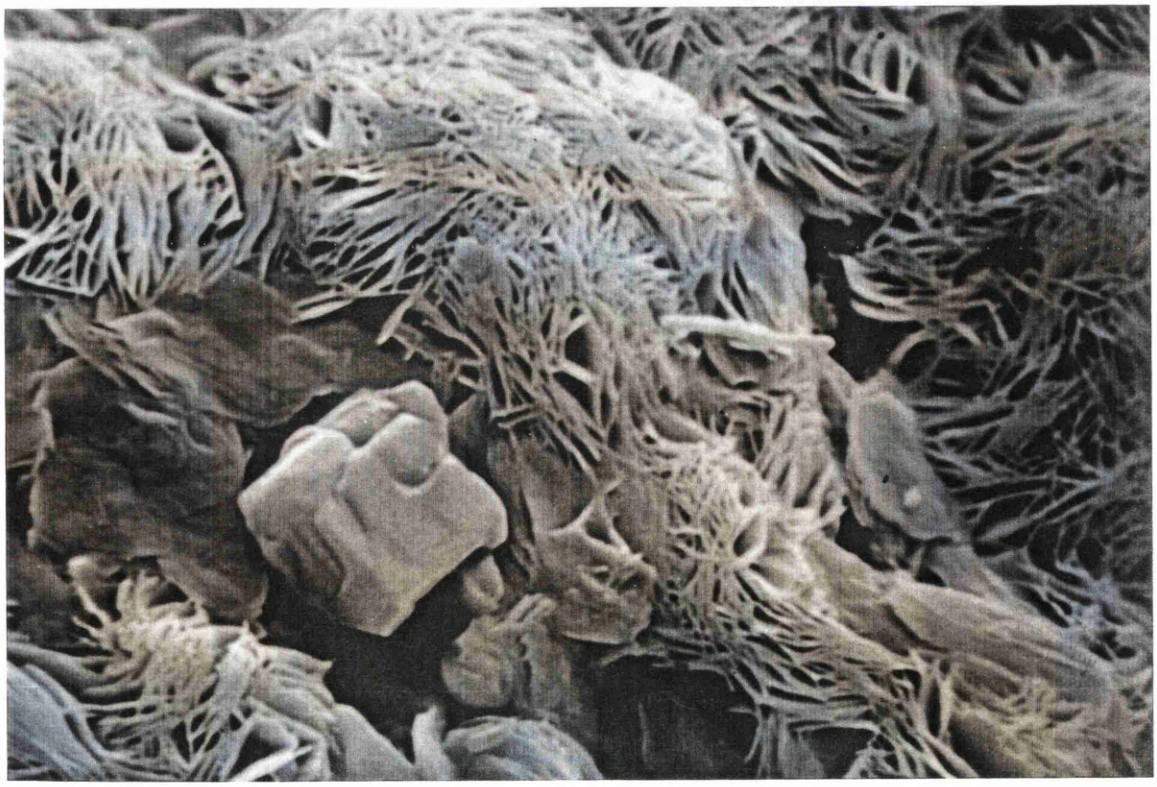


Figure 9.3.6. *C.caretta*, a non-viable eggshell, x 2500.

Figure 9.3.7. *C.mydas*, clutch 1, the typical aragonite crystals with occasional calcite blocks (arrow), x 1250.

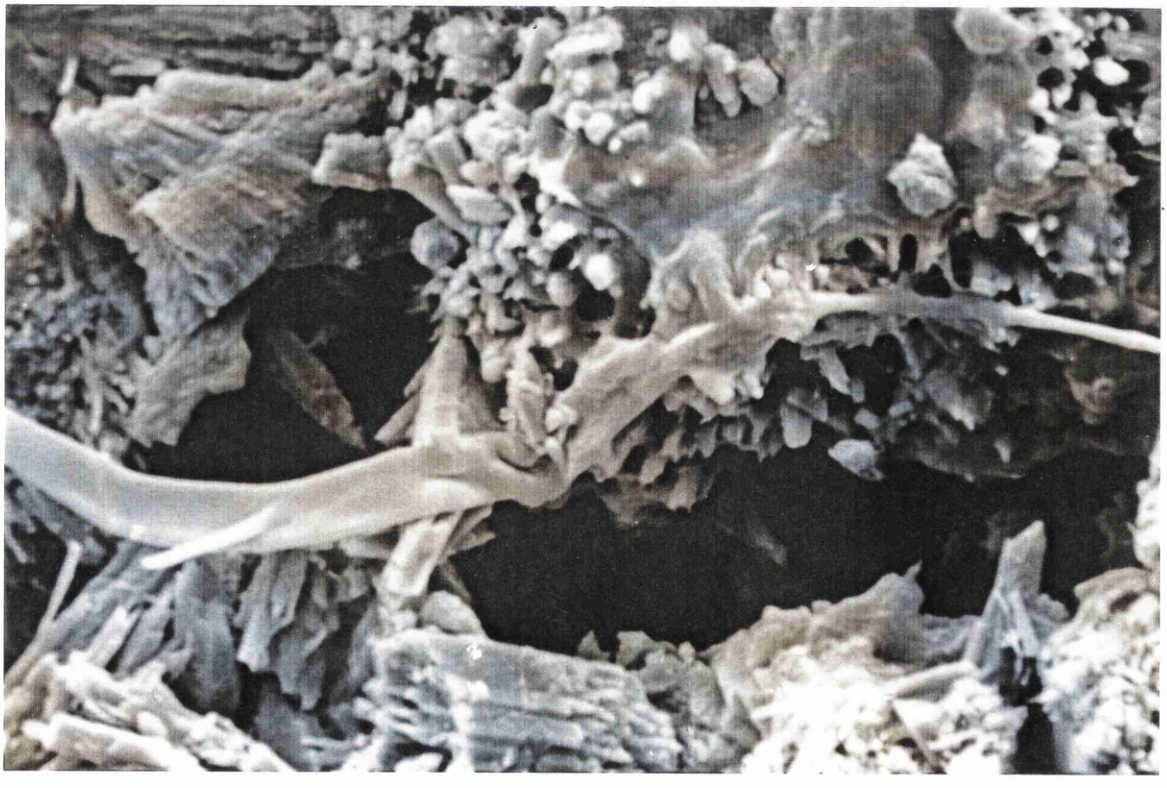


Figure 9.3.8. *C.mydas*, clutch 1. Mucilaginous covering over the spicular aragonite of the eggshell, x 1250.

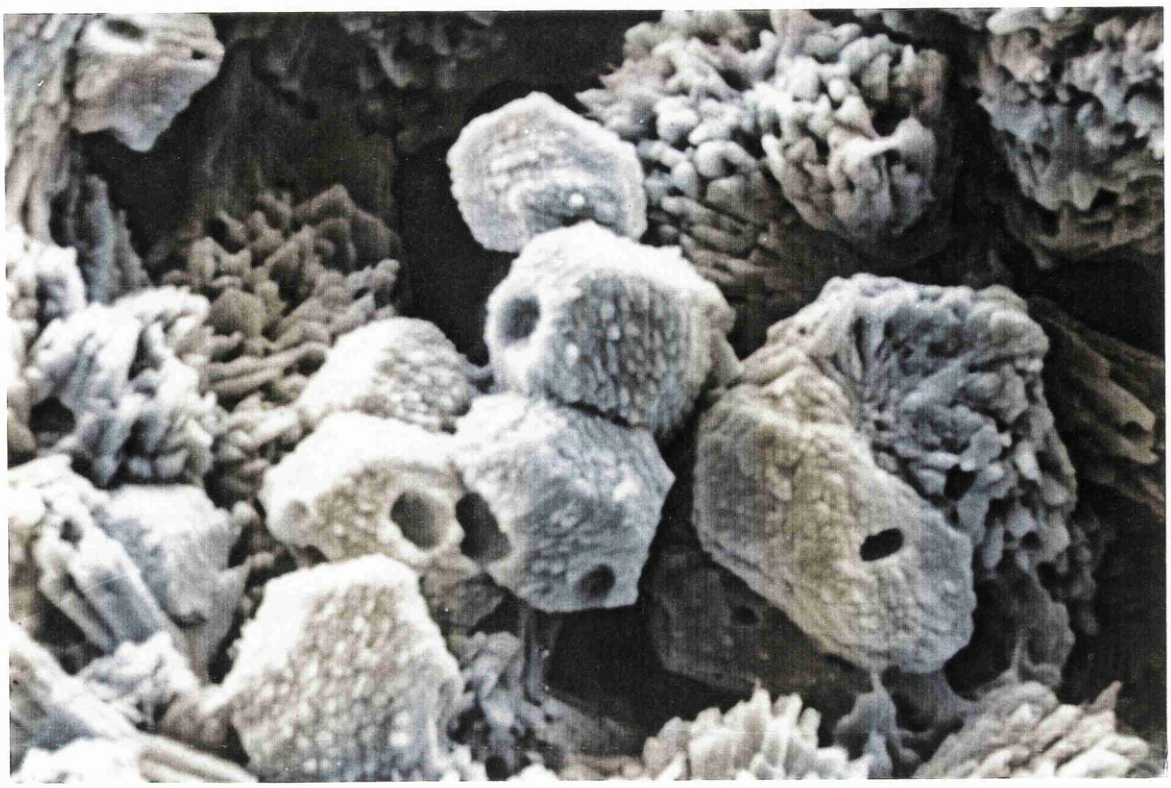


Figure 9.3.9. *C.mydas,* clutch 1. Calcite, x 1250.

Figure 9.3.10. *C.mydas,* clutch 1. At this magnification cubic crystal growth can be seen within the larger crystal mass, x 5000.

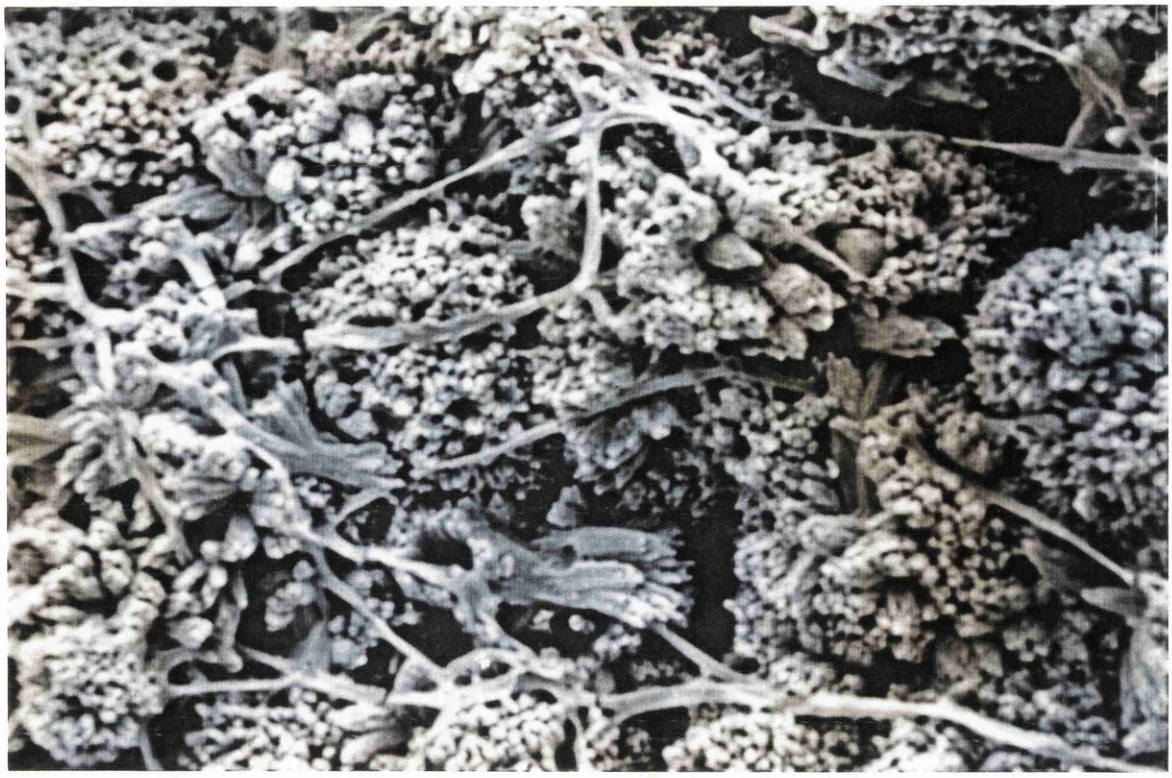


Figure 9.3.11. *C.mydas,* clutch 1. Fungal hyphae forming a web over the outer crystalline layer, x 320.

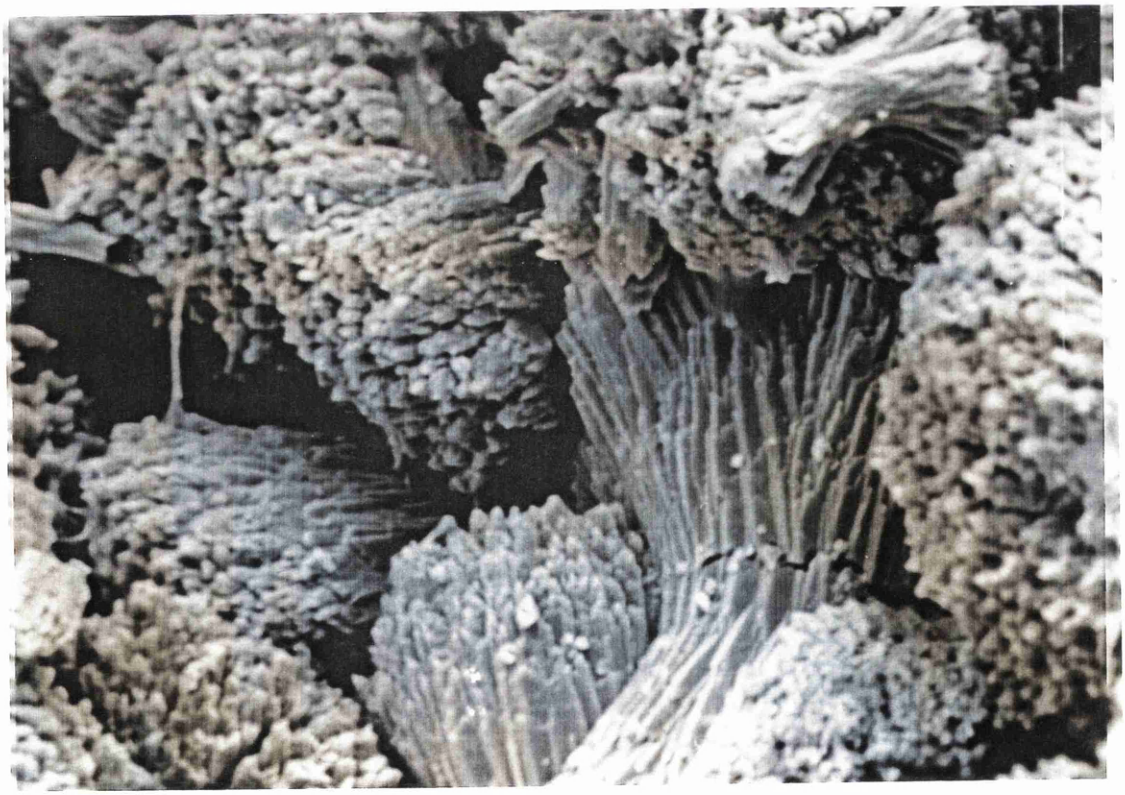


Figure 9.3.12. *C.mydas* clutch 2. The normal arrangement of spicular aragonite crystals, with their wheatsheaf appearance, x 1250.

Figure 9.3.13. *C.mydas* clutch 2. Blocks of calcite (arrow) are still present in eggshells from the middle of the laying season, x 640.

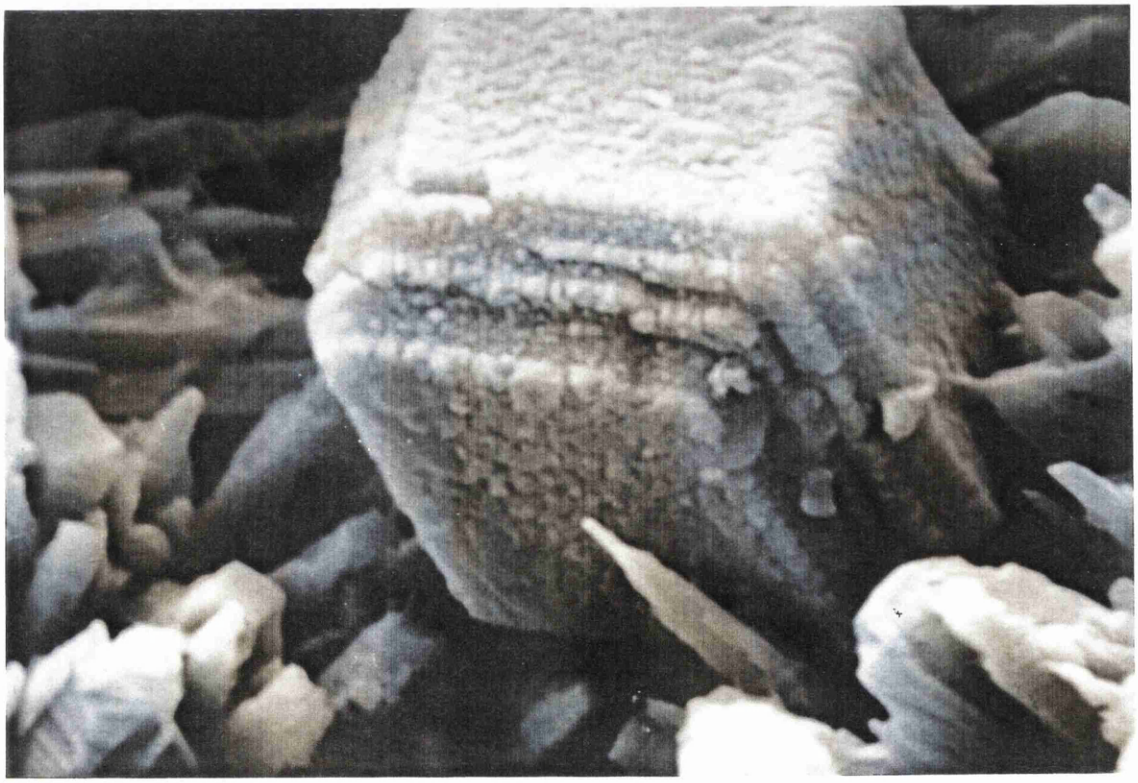


Figure 9.3.14. *C.mydas* clutch 2. Higher power micrograph of figure 9.3.13., x 2500.

Figure 9.3.15. *C.mydas* clutch 3. The sharp spicular arrangement has been lost and the shell is taking on a molten appearance, x 1250.

Figure 9.3.16. *C.mydas* clutch 4. The eggshell displays regions of normality, x 320.

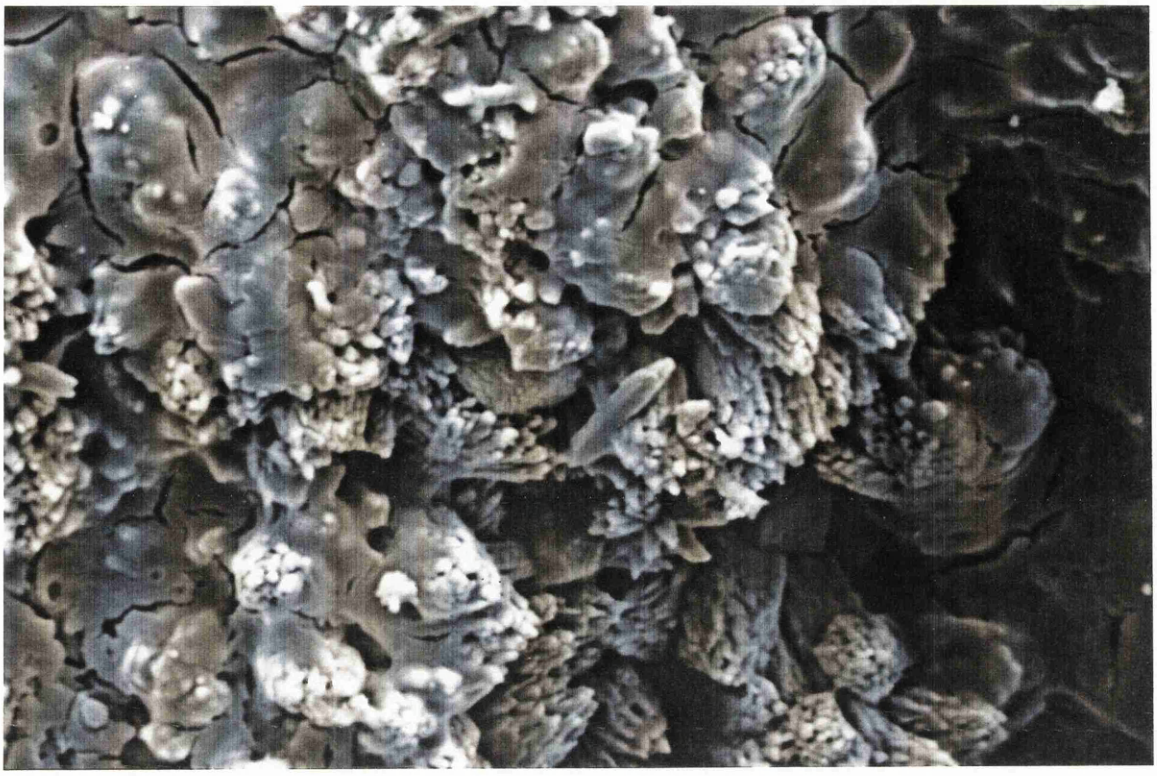


Figure 9.3.17. *C.mydas* clutch 4. An amorphous deposit obscures the aragonite crystals, x 640.

Figure 9.3.18. *C.caretta* clutch 1. Aragonite crystals on the surface of the eggshell, x 1250.

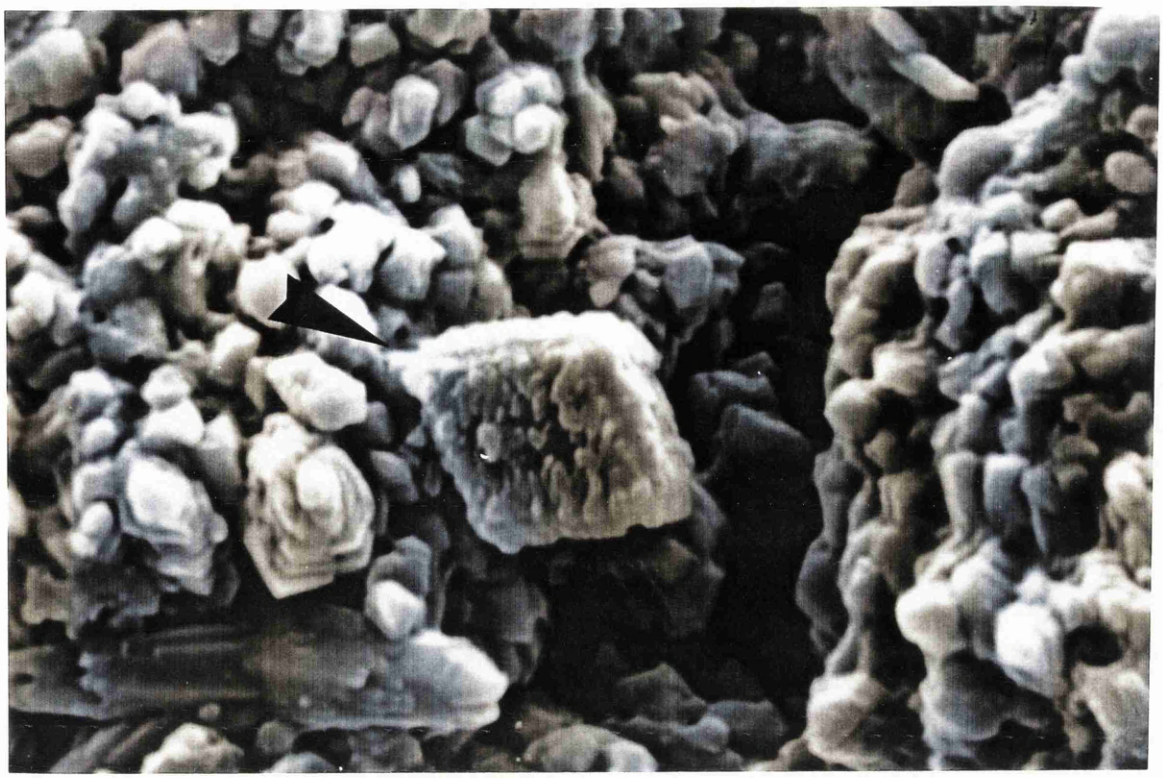


Figure 9.3.19. *C.caretta* clutch 1. Calcite blocks (arrow) are present in association with aragonite crystals at this stage, x 2500.

Figure 9.3.20. *C.caretta* clutch 1. The nodular arrangement is particularly well illustrated in this micrograph, x 320.

Figure 9.3.21. *C.caretta* clutch 1. Lozenge shaped crystals, x 2500.

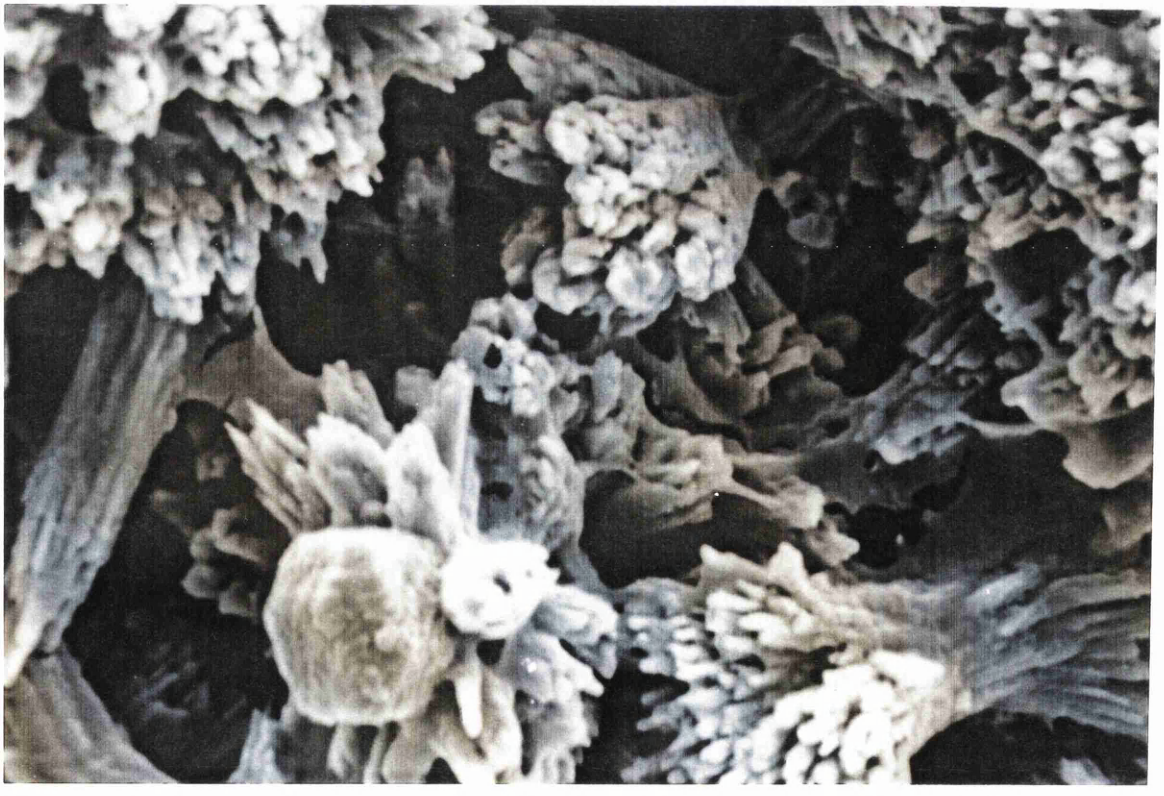


Figure 9.3.22. *C.caretta* clutch 2. Aragonite and calcite exist on the surface of the eggshell, x1250.

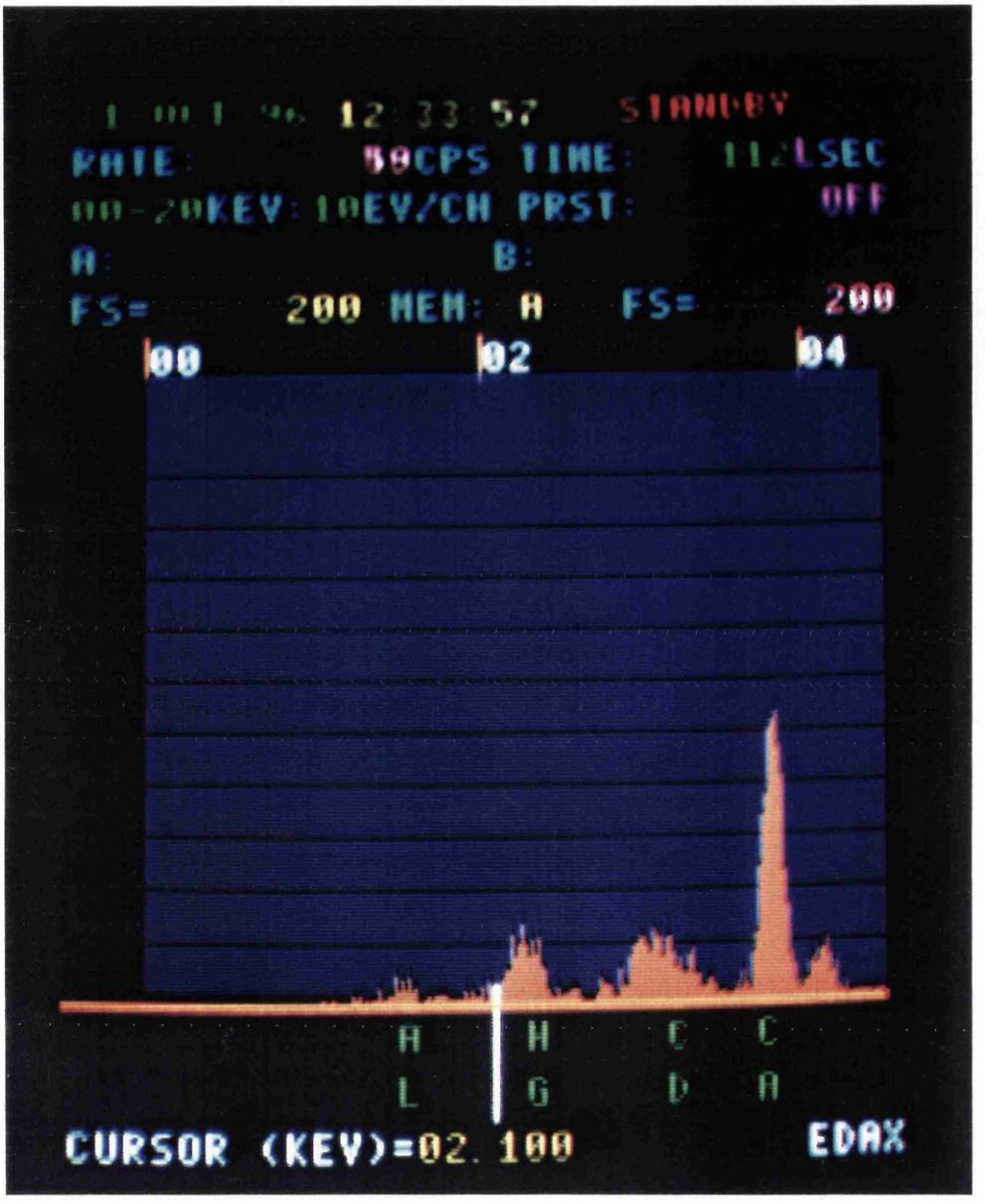


Figure 9.3.23. Spectrum obtained from microanalysis of a coated *C.caretta* eggshell.

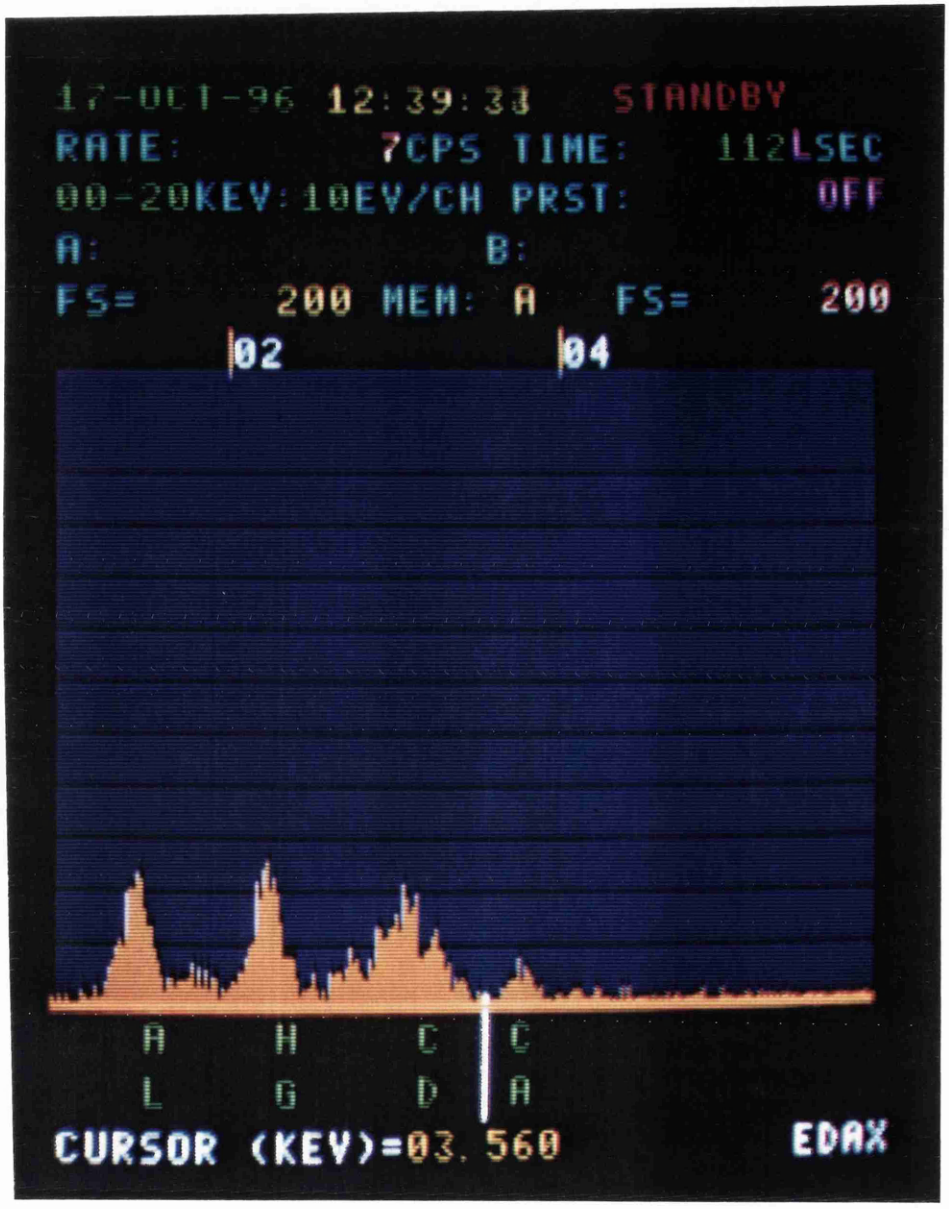


Figure 9.3.24. Spectrum obtained from microanalysis of a coated *C.mydas* eggshell.

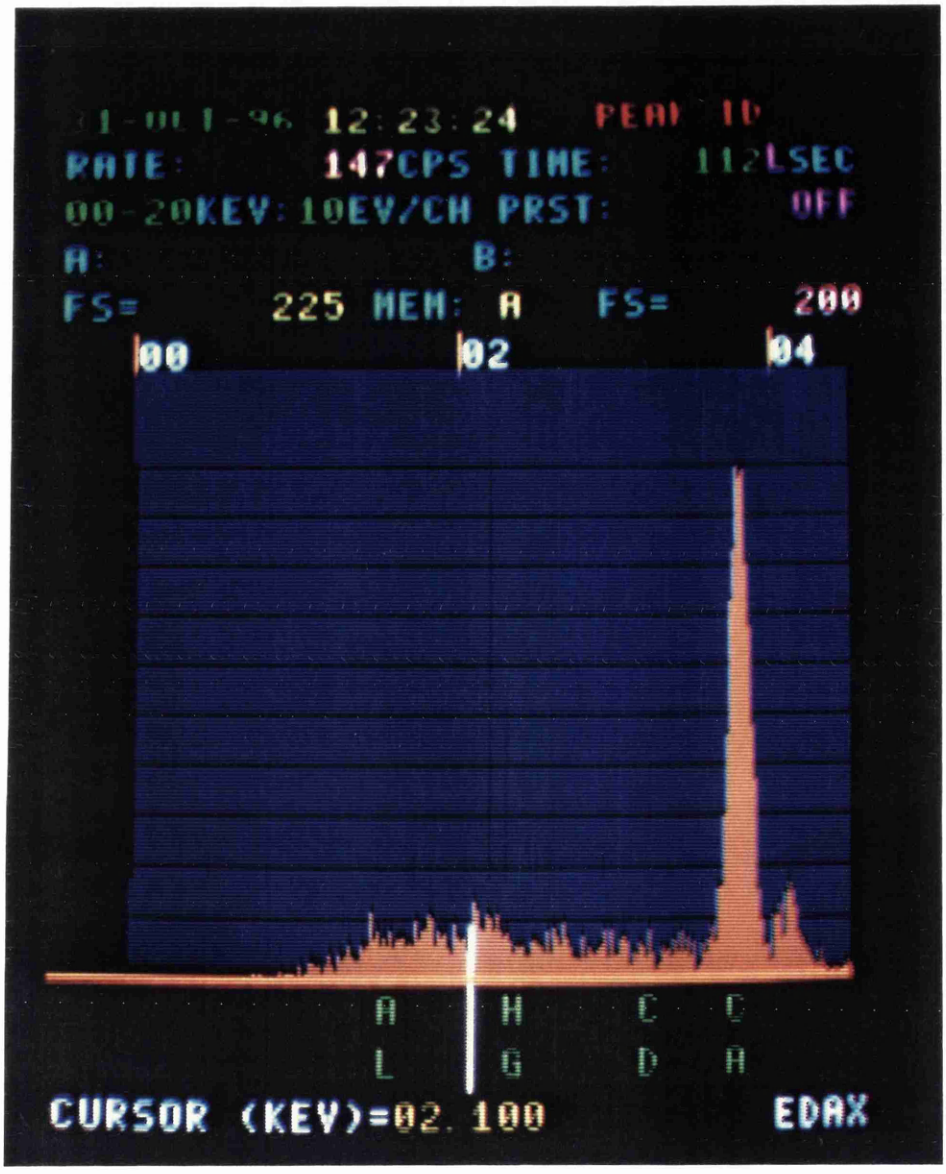


Figure 9.3.25. Spectrum obtained from microanalysis of an uncoated *C.caretta* eggshell.

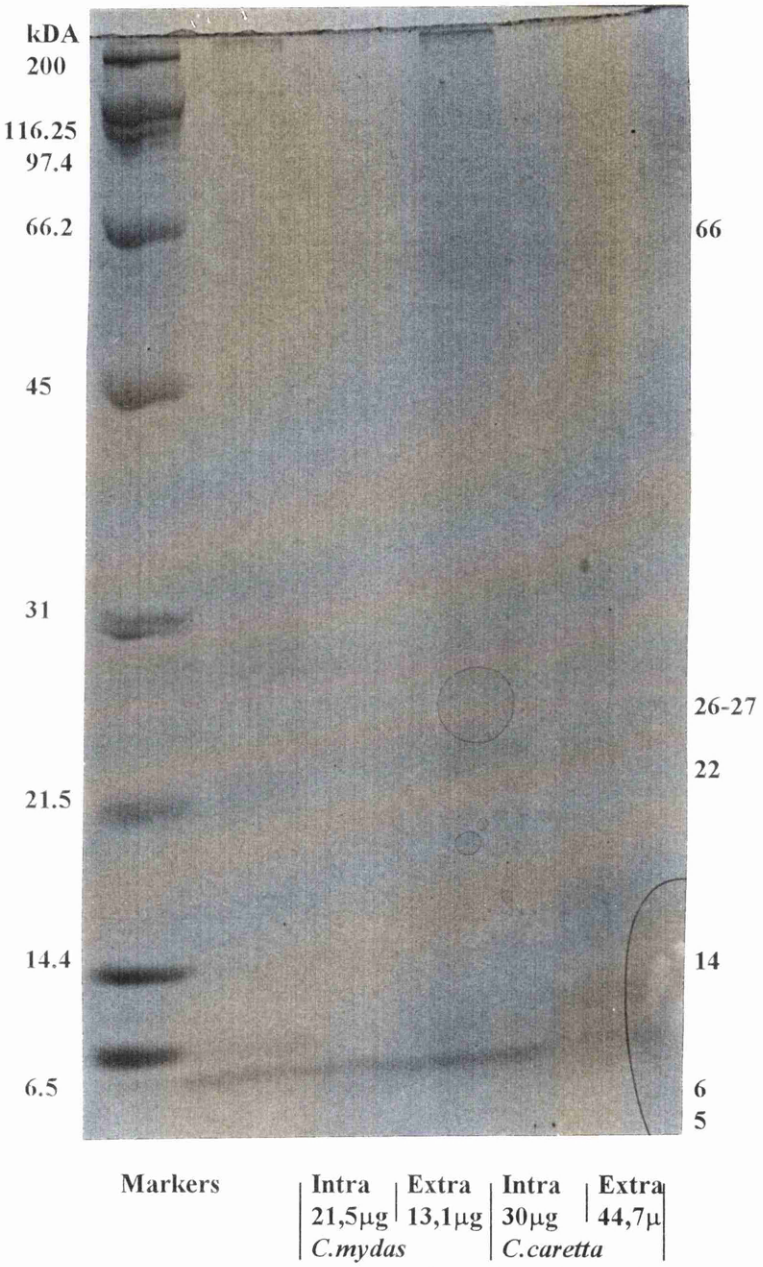


Figure 9.3.26. Intra and extra mineral protein fractions of eggshells of *C.mydas* and *C.caretta*.

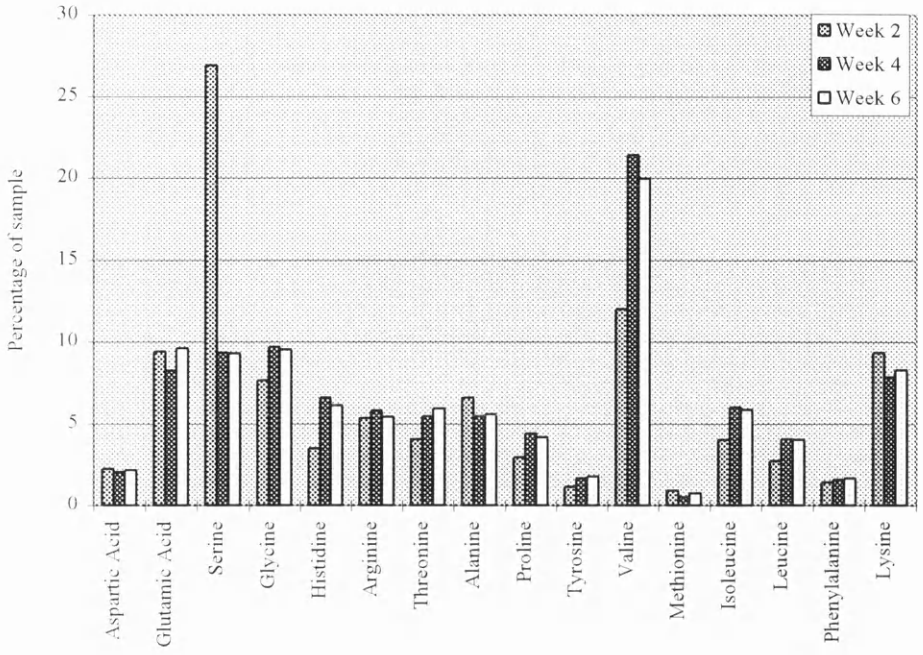


Figure 9.3.27. Comparison of amino acid levels found in *C.mydas* egg shell, 1994.

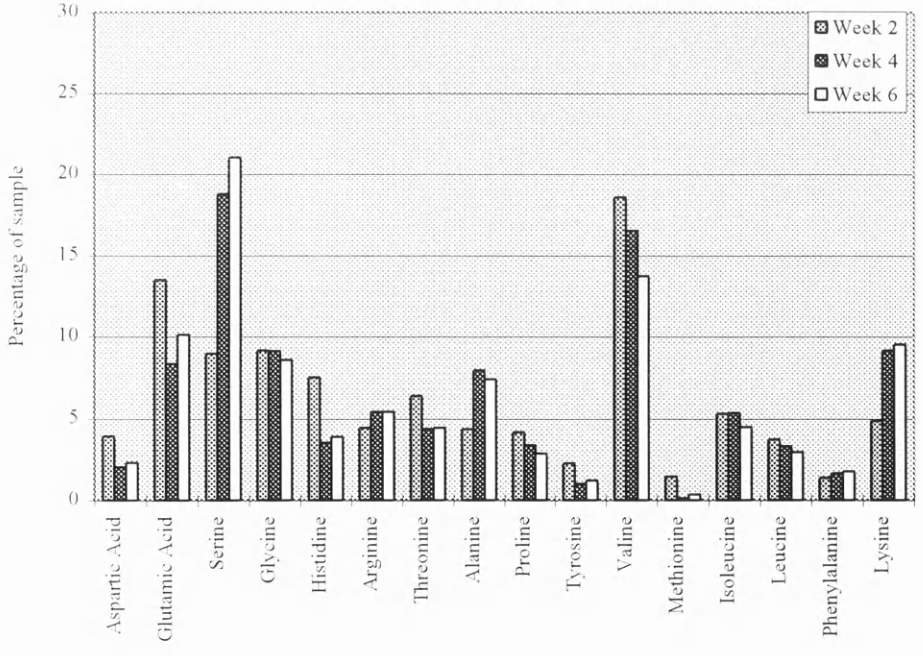


Figure 9.3.28. Comparison of amino acid levels found in *C.caretta* egg shell, 1994.

9.4. Discussion

The morphological variations observed within the inorganic aspect of the eggshell of *C. mydas* and *C.caretta* are in accordance with those reported by Baird and Solomon (1979) and Solomon and Watt (1985). In both species, aragonite, the main polymorphic form of calcium carbonate, co-exists with calcite, indicating some physio/chemical change in the oviducal environment, not necessarily associated with a reduced availability of calcium. A supersaturated solution of $CaCO_3$ is required for crystal formation. As the embryo withdraws calcium from the aragonite structures, the latter become more fragmented as observed in this series of experiments and reported earlier by Solomon and Tippett (1987).

At hatching, calcite blocks remain intact, unaffected by the process of dissolution. At best they are a waste of $CaCO_3$ and at their worst, in situations where they dominate the crystal pattern, they can reduce hatchability (Solomon & Tippett 1990). The co-existence of the inorganic fraction with an organic moiety is not a new concept.

To date, five functional properties have been attributed to the organic matrix as it exists within the avian eggshell, these are; a reinforcing network, crystal nucleation, crystal orientation, control of crystal morphology and the control of the polymorphic stage. Gautron *et al.* (1993) and Fraser (1996) in a series of experiments have demonstrated, by gel separation, the protein profiles of the soluble and insoluble fractions of the eggshell, and illustrated the role played *in vitro* by shell matrix extracts and the growth of calcium carbonate.

The nucleation and growth of the avian eggshell is quite different from that observed in most reptilian species insofar as the membrane fibres, onto which the calcium salts deposit, appear to exert an influence on crystal growth. These fibres also bond with the shell, ensuring a firm foundation for the formation of the multi-layered testa. The process of plasma etching described by Reid (1983) is used to remove the membrane fibres of the hen's egg without disrupting the inorganic fraction of the shell. Fraser (1996) comments that following etching, the 35, 43 and 50 kDa levels are removed, i.e. they are membrane associated. Their absence, in the present situation, is perhaps therefore not surprising. The organic/inorganic fractions of the turtle eggshell do not bond. The spicular aragonite crystals deposit onto saucer-shaped nucleation sites formed at the end of membrane release. It is this loose attachment which assists in the process of shell swelling as the egg absorbs moisture

from the sand. In this respect it is also of interest to note the absence of the 17 KDa band in the turtle eggshell matrix. This band, identified by Hincke *et al.* (1995) as ovocleidin, is also crucial in the early stages of avian eggshell formation, where it appears to be associated with both the shell membranes and mamillary cores.

Of the remaining bands, the domestic fowl and turtle appear to share at least two in common, i.e. the 66 KDa and 21-22 KDa bands. It is feasible that the hypothesis put forward by Fraser (1996) regarding the possibility that these are subunits of the keratan and dermatan sulphates identified by Arias *et al.* (1992) is also true for the turtle eggshell. On the other hand, sharing a numerical value does not necessarily mean that the same proteins are present in both species. Given the absence of many of the bands observed in the avian eggshell, and the quite different morphology of their separate eggshells, it is more than likely that the matrix proteins in the turtle eggshell are unique to this situation.

The results of the amino acid analyses do not help in discerning the nature of the proteins present, but they do support the idea that the observed changes in crystal morphology, reported herein, are accompanied by simultaneous changes in the nature of the proteins present as judged by the proportional differences in concentration of amino acids. In order to resolve and compare the nature of the proteins present within the crystal layer of the eggshell of turtle and hen it would be necessary to conduct a series of end terminal analyses (gel electrophoresis).

The hatching success of chicks depends on the age of the bird from which the egg originated, the quality of the shell she produced, and the hatching environment. Solomon (pers. comm) reports that the percentage hatch rate increases from 22 weeks of age to 40 weeks of age and thereafter declines. The values follow the observed pattern for improvement in shell quality. The latter peaks at 35-40 weeks of bird age.

It would appear from the present results that the turtle shows a similar trend for improved shell quality and hatching success (chapter 4) mid-season and thereafter demonstrates a higher inclusion of shell defects which may impede embryonic development. The increase in defects may be interpreted as a reflection of a depletion in resources. The different dietary requirements of the species will undoubtedly reflect on the ease with which the depletion becomes manifest.

This chapter has posed more questions that it has answered - but research is by its very nature an ongoing activity in which new technology is constantly modifying previous findings. What is does do however, is underline the fact that the micro-environment provided by the eggshell can also be hostile. Whilst conditions within the nest may be ideal for incubation and development of embryos if the eggshell is inadequate other variables are irrelevant.

Chapter 10 - General Discussion and Recommendations

This thesis originated form a public expression of concern for the perceived decline in the numbers of marine turtles nesting annually on the shores of Northern Cyprus. Data collected prior to the present investigation were sporadic and incomplete, but nevertheless served to indicate that this small island had a unique role to play in the reproductive effort of both *Chelonia mydas* and *Caretta caretta*. With respect to the species in question, it is the most comprehensive study of its type to have been conducted in the Mediterranean. It has also provided a unique opportunity to compare the two species and their nesting habits simultaneously at the same study site.

Some of the results herein suggest that marine turtles nesting in Cyprus share similar traits with conspecific populations nesting outwith the Mediterranean, for example; the inter-nesting intervals of females, the number of clutches they lay in one season, the depth of their nests, the particle size of the sand in which they nest and their hatching and hatchling emergence success. With reference to size however, *C.caretta* nesting in the Mediterranean have been shown to be significantly smaller than those nesting outwith (Dodd 1988; Erk'akan 1993; Margaritoulis 1989; Sutherland 1985). Similar observations were recorded for this species in Northern Cyprus. These smaller individuals also laid significantly smaller clutches compared to those recorded in both Greece and Turkey. *C.mydas* females nesting in the Mediterranean, whilst not significantly smaller than those nesting outwith, are at the lower end of the range of sizes recorded nesting for this species.

Some of these variations may be a result of the genetic distinction of these populations, which is considered by many to reflect the relative reproductive isolation of these species within the Mediterranean basin. In addition, varying environmental conditions, in particular temperature and food availability, are likely to have an effect on the biology of these species. Certainly temperature has been shown to affect the incubation periods of nests, with those recorded in Cyprus being significantly shorter than those recorded elsewhere in the Mediterranean (Erk'akan 1993; Gerosa *et al.* 1995; Margaritoulis 1989; Peters & Verhoeven 1992; Sutherland 1985; van Piggelen 1993).

Whilst some of the information presented in this work has been described for other nesting populations, some aspects have only been sparsely examined on a world-wide basis. In particular, whilst many individuals have studied the role of temperature on nest incubation and embryonic development, few have examined in any detail the temperature of *in situ* nests. In the present study, temperatures within such nests ranged between 28.5 and 33 °C

in *C.mydas* nests and 28.1 and 33 °C in those of *C.caretta*. In nests of both species, the majority of the incubation period was spent at temperatures above 29°C, which has been shown in studies outwith the Mediterranean to be the pivotal temperature above which the sex ratio becomes increasingly skewed towards a greater proportion of females (Harry & Limpus 1989; Mrosovsky *et al.* 1995). Thus, although sample sizes were small, this study suggests either that 29 °C is not the pivotal temperature for these species in Cyprus or a greater number of hatchlings produced in Cyprus are female. However, seasonal and diurnal variations in the temperatures recorded in these nests may result in a different sex ratio than achieved through artificial incubation at these temperatures. In light of the issue of global warming, interesting questions arise as to whether these species will be able to adapt to such climatic changes. Findings in this study, however, indicate that the onset of the nesting season is variable and may be governed by temperature and thus, if global warming does occur, these populations may be able to shift their season or nesting location so that it coincides with the correct temperatures for successful incubation.

Information concerning the nesting behaviour of these species is sparse. This study demonstrates that, on the same beach, *C.mydas* females spend, on average, twice as long in completing the nesting process in comparison with *C.caretta*. Indeed, the average *C.mydas* female takes two and a half hours to complete nesting. This is mainly a result of the longer time spent covering the body pit. In *C.mydas,* a negative relationship was recorded between the time spent digging the egg chamber and the hatching success and hatchling emergence success of the nest. This may reflect an unsuitable nesting substrate with suboptimal conditions for embryonic development leading to a reduced level of success. This relationship was not recorded for *C.caretta,* nevertheless, individuals of this species laying larger clutches took a longer time to do so, a relationship that might have been expected although was not demonstrated for *C.mydas*.

In recent years an attempt has been made to address the possible effect of observer disturbance on nesting females (Campbell 1994; Johnson *et al.* 1996). None however, have assessed the effect of tagging females on their subsequent nesting behaviour. In this study, there was no evidence that tagging females influenced either their behaviour or the resultant success of the nest. It is important for researchers to quantify any deleterious effects that their studies might have, particularly in the case of an activity such as tagging which involves such close contact and may cause some discomfort to the animal.

Most researchers have failed to consider how crucial the quality of the egg and shell are to the delicately balanced life cycle of marine turtles. As with birds, the shell has a number of roles to play during the incubation period. The present pilot study highlights the inclusion of structural defects in the shell towards the end of the laying season. These changes in crystal structure have been reported previously in the domestic fowl and correlated with failure to hatch. Perhaps of greater significance however, are the concomitant changes in the levels of amino acids within the crystalline framework of the shell. The role of the protein matrix in regulating calcium deposition is the subject of current research (Bain pers. comm.) and the results reported herein highlight possible areas of research towards a greater understanding of the factors underlying aragonite and calcite deposition.

Prior to this study, the size of the populations of marine turtles nesting in the Mediterranean were estimates based upon scant biological knowledge (Groombridge 1990). Indeed, these estimates applied the same criteria to the two species which clearly differ in their reproductive strategies. To date, 60 *C.mydas* and 99 *C.caretta* females have been tagged at Alagadi (1992-1996), allowing information to be gathered as to their favoured remigration and inter-nesting intervals, and so leading to more accurate estimates of the nesting populations in this region.

The result obtained from this tagging programme indicate that whilst *C.mydas* were recorded as laying a mean of 2.4 clutches in any one season, *C.caretta* females were only as laying 1.6 clutches in the same period. The latter species also require a slightly longer period between laying subsequent clutches (13.5 days) compared to *C.mydas* (13 days). However, whilst some *C.caretta* females are able to lay in consecutive seasons, and others favour intervals of 2 or 3 years equally, *C.mydas* females have only been recorded remigrating after 2 or 3 year intervals. These findings are, however, only based on 5 years of data and a maximum possible remigration interval of 4 years. Some females nesting at this site may require a longer period between nesting seasons to replenish their reserves to levels which enable egg production. It is, therefore, crucial that this study be continued for at least a further 5 years to discover whether this is the case. This study does, however, underline the importance of this nesting site in Northern Cyprus given the extremely low estimated number of *C.mydas* females nesting annually in the Mediterranean (300-500 annually; Groombridge 1990).

The size of the populations calculated by Groombridge (1990) were based on the assumption that each female laid, on average, 3 clutches in a given season. Whilst this study has shown that this may indeed be a close approximation for *C.mydas* females nesting in Cyprus, *C.caretta* lay fewer clutches and hence the estimate of 2000 females nesting annually is likely to be an under-estimate. Several other methods of estimating population size are described in this thesis which it is believed lead to a more accurate value. This information, however, should be treated with caution if applied to other populations nesting in the Mediterranean as this study has clearly illustrated how such populations vary in different regions of this relatively enclosed sea.

The inter-species variations recorded in nesting frequency may be a consequence of the larger size of *C.mydas* nesters in comparison to the smaller *C.caretta*, and the strong relationship recorded between female size and mean clutch size. Although in both species, larger females laid larger clutches, they did not lay significantly more clutches in one season than smaller females. However, females of both species, nesting earlier in a season were recorded to lay a greater number of clutches, but whilst larger *C.caretta* females were amongst the first females in a season to nest, this was not the case for *C.mydas*. In the latter species, females that required a shorter inter-nesting interval ultimately produced a greater number of clutches in a season. *C.mydas* females also laid deeper nests and produced larger hatchlings than *C.caretta*, and both of these variables increased with the size of the female in the former species. No such relationships were recorded in *C.caretta*, however nests of larger *C.caretta* females were found to have shorter incubation periods.

C.caretta nests which produced larger hatchlings had a greater hatching and hatchling emergence success. This would indicate that, in this species at least, the fitness of hatchlings is related to their size. Thus, larger hatchlings are able to emerge from both the egg and nest more successfully than smaller individuals. Alternatively, it may be that the site selected by the female had more optimal conditions for development leading to a greater hatchling fitness and thus a higher hatching and hatchling emergence success. The latter features were also recorded in deeper nests with respect to the depth to the top of the nest but not between the depth to the bottom of the nests and hatchling emergence success. Thus whilst deeper nests may have greater protection from adverse environmental conditions and consequently a higher hatching success, a greater depth may make it harder for hatchlings to crawl up through the sand resulting in a higher incidence of hatchling mortality.

As in many studies of this nature, it must be remembered that the inter-species variations in nesting behaviour may result in a bias in the data recorded. The shorter time in which *C.caretta* females take to complete their nesting process might result in a smaller proportion of the nests of this species being observed. Added to this is the fact that track and pit morphology is a less dependable assessment of nesting activity than direct observation and so the number of hatched nests recorded for this species may not be as accurate.

Recommendations

C.mydas nests were, on average, more successful that those of *C.caretta*. It is likely that this is a result of nests of the latter species being shallower and in closer proximity to the sea. In addition, both predation and insect infestation are higher in nests of this species. At this stage of the life-cycle and with the correct management, many of the factors having a detrimental effect on the success of *C.caretta* nests can be reduced. Protection of nests either by relocation or placing screens above them, increases the number of hatchlings produced from nests on the Alagadi Beach each year. Such relocation activities have been shown to increase the number of nests which successfully hatch, however both the temperature and sand quality of *in situ* nests should be studied in greater detail so that relocated nests are incubated in conditions more suitable for successful development.

Control of beach users is also an issue with respect to both adult and hatchling turtles. The Department of Environmental Protection in Cyprus has undertaken to decrease human pressure at this site by restricting access to the beach at night and to certain areas of the beach during the day. The large numbers of people using this beach during the summer months leave behind them copious quantities of litter. In addition, due to the polluted nature of the Mediterranean, large volumes of marine litter are frequently washed ashore onto this beach during the summer months. This can obstruct both adult and hatchling marine turtles and may influence the temperature of incubating nests. On several occasions both nesting adults and emerging hatchlings have been found trapped beneath beach debris. In some cases this has been fatal. In addition, an increase in organic materials may attract predators to the area resulting in a greater loss of nests. A strict cleaning regime is needed, which although implemented is not always fulfilled.

In the first year of this study large amounts of sand were extracted from Alagadi Beach. The regulations permitting sand only to be removed from behind existing dunes were not adhered to. Large scale removal was eventually stopped although extraction still occurs at a minor level. It has however had an effect on the beach with some areas becoming too stony and shallow for females to nest.

Although fishing is mainly recreational around the Alagadi area, much of this is by spear gun. On one occasion a nesting female emerged with a spear (1.3m), from such a gun, deeply piercing her carapace. This was removed by an assisting veterinary surgeon, but the prognosis for survival was deemed poor.

All of these issues can in some way be improved by a public information programme being conducted by members of GUTCE, but this should also be undertaken by local authorities as a long term policy.

If the volume of literature is a reflection of interest, then the enigma surrounding the breeding cycle of marine turtles has fascinated both layman and scientist for hundreds of years. Anecdote has been translated into fact and the breeding strategies of many populations of marine turtles have been documented for nesting beaches from Turkey to Tortuguera. For a migrating species each investigation must be regarded as a snap shot - a chance to record the facts as they exist at that moment in time. Whether the complex process of nesting and hatching are the same in subsequent years cannot be predicted, but the possibility exists, on the relatively unused shores of Northern Cyprus, to maintain an environment which will afford these species continuity in their reproductive effort for many years to come.

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